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ARMSTRONG LABORATORY

# PHARMACOKINETICS OF HCFC-123 IN DOGS

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## TECHNICAL REVIEW AND APPROVAL

**AL/OE-TR-1995-0025**

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

**FOR THE COMMANDER**

**TERRY A. CHILDRESS**, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

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The U.S. EPA has been mandated to evaluate alternatives to Class I ozone depleting substances including halon fire and explosion protection agents. Under the Significant New Alternatives Policy Program the EPA considers the cardiac sensitization potential as the most sensitive biological endpoint because of the potent sensitizing effect, in the epinephrine-challenged dog model, of many of the halogen containing chemicals and replacement candidates of this program. This study was initiated to evaluate the pharmacokinetic behavior of HCFC-123 in an exposure scenario that mimics the cardiac sensitization test in dogs. Specific emphasis was placed on the measurement of blood and tissue samples following exposure of 1 or 5% HCFC-123 for one to five minutes duration.

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## PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit (THRU), ManTech Environmental Technology, Inc., located at Wright-Patterson Air Force Base, OH. Work at the THRU is performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F08 and F33). Lt Col Terry A. Childress served as the Contract Technical Monitor for the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory. This document serves as a final report on the pharmacokinetics of HCFC-123 in dogs. The research described herein began in October 1993 and was completed in May 1994. Part of this research effort was carried out at WIL Research Laboratories, Inc, located in Ashland, OH.

The animals used in this study were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

The authors thank R.K Black, M. Caracci, J.R. Creech, and S.K. Neurath for their assistance in collecting the blood and tissue samples during the course of the pharmacokinetic studies.

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## ABBREVIATIONS

| | |
|---|---|
| CFC | Chlorofluorocarbons |
| cm | Centimeter |
| $EC_{50}$ | Effective concentration producing effect in 50% of test animals |
| HCFC-123 | 1,1-Dichloro-2,2,2-trifluoroethane |
| L | Liter |
| lb | Pound |
| m | Meter |
| $\mu$l | Microliter |
| mg | Milligram |
| mm | Millimeter |
| min | Minute |
| NOAEL | No observable adverse effect level |
| ppm | Parts per million |
| psi | Pounds per square inch |

## SECTION 1

## INTRODUCTION

HCFC-123 is used primarily as a foam blowing agent, as a refrigerant, and in cleaning solvents. The Air Force is considering the use of HCFC-123 as a fire extinguishant, replacing Halon 1211. Halon 1211 has been used as a fire extinguishant in streaming systems, where the extinguishant is manually discharged through a nozzle of small, portable units that are commonly found in industry, military, and office settings. Jarabek et al. (1994) reviewed the process of searching for chlorofluorocarbon substitutes, with HCFC-123 as a specific example.

Potential occupational military exposures with HCFC-123 as a fire extinguishant include maintenance personnel (crew chiefs) responding to aircraft fires on the flight line or in a large indoor structure, such as an aircraft hangar, and trained fire fighters responding to alarms. The fire fighter exposure scenario deals with military personnel who don appropriate fire fighting gear, including respirators, immediately prior to fighting fire. Thus, the exposure scenario of concern involves the emergency situation where maintenance personnel attempt to put out a fire without availability of the appropriate fire fighting equipment. The exposure duration of concern involves a 1-minute period to simulate personnel discharging either the entire contents of a small (1- or 3-lb) extinguisher or the partial contents of a large (150-lb) extinguisher while attempting to put out an aircraft fire (usually an engine fire) from upwind of the fire (Dr. Kibert, WL/FIVS, personal communication).

The U.S. EPA has been mandated under Title VI of the U.S. Clean Air Act of 1990 (Public Law 101-549) to evaluate alternatives to Class I ozone depleting substances, including halon fire and explosion protection agents. Under the Significant New Alternatives Policy Program the EPA considers the cardiac sensitization potential as the most sensitive biological endpoint because of the potent sensitizing effect of this chemical and similar chemicals in the epinephrine-challenged dog model. For HCFC-123, the $EC_{50}$ (95% confidence interval) was determined by Trochimowicz and Mullin (1973) to be 1.9% (1.29% to 2.82%) for a 5-minute exposure. Dogs exposed to 10,000 ppm for 5-minute show no "marked" arrhythmias, and this can be considered to be the no observable adverse effect level (NOAEL). Converting this 5-minute NOAEL to a 1-minute NOAEL is not straightforward. Haber's Law (Haber, 1924) suggests that the 1-minute NOAEL would be 5%, because the product of concentration and time is a constant. Haber's law is applicable to a limited number of situations in which all processes are linear and the toxic moiety is the parent chemical.

The objective of this study was to evaluate the pharmacokinetic behavior of HCFC-123 in an exposure scenario that mimics the cardiac sensitization test in dogs. Specific emphasis was placed on the measurement of blood and tissue samples following exposure of 1 or 5% HCFC-123 for 1 to 5 minutes duration.

## SECTION 2

## MATERIALS AND METHODS

### TEST MATERIAL

1,1-DICHLORO-2,2,2-TRIFLUOROETHANE (HCFC-123)

Physical and Chemical Properties

Chemical Formula: $CHCl_2CF_3$

Molecular Weight: 152.9

Synonyms: HCFC-123

CAS No.: 306-83-2

Physical state: Liquid at normal temperatures

Boiling point: 27.9 °C @ 760 mm Hg

Freezing point: -107 °C

Vapor pressure: 11 psi (20 °C)

Vapor density: (Air = 1) 3.6

Solubility in water: 0.21% (wt) @ 70 °F

Flash point: N. A. - No flash point

Auto ignition: Unknown, probably not applicable

Flame limits: (In air, % by vol), none

**General Procedure**

Two male beagle dogs per time-point were exposed to HCFC-123 at either 1% or 5% for various exposure durations (Table 1). Exposure was "nose-only" via a specially adapted canine anesthesia mask equipped with a two-way non-rebreathing valve. The exposure system was designed to provide instantaneous exposure of the dogs to the target concentrations and permit the drawing of blood samples. Blood samples were collected (as applicable) at 0 (pre-exposure), 1, 2, 3, 4, 5, 7.5, 10, 15, 30, 45, and 60 minutes (during exposure), and 1, 3, 6, 16, and 31 minutes (postexposure recovery) and analyzed for HCFC-123. At the end of the exposure periods (or postexposure recovery periods), animals were euthanized and samples from selected tissues (heart, liver, fat, and skeletal muscle) were collected as rapidly as possible for analysis of HCFC-123.

**Table 1. Experimental Design**

| Number of Dogs | Dog I.D. Number | Exposure Concentration (HCFC-123) | Exposure Time | Postexposure Time |
|---|---|---|---|---|
| 2 | 1974<br>1999 | 1% | 1 min | na* |
| 2 | 1975<br>1986 | 1% | 5 min | na |
| 2 | 1992<br>1995 | 1% | 60 min | na |
| 2 | 1993<br>1994 | 1% | 60 min | 30 min |
| 2 | 1979<br>1990 | 5% | 1 min | na |
| 2 | 1983<br>1997 | 5% | 5 min | na |

*na = not applicable

**Exposure System**

Liquid HCFC-123 was evaporated by heating a glass reservoir while air passed across the test article surface. The HCFC-123 was first brought to target concentrations in a 500-liter NYU-type inhalation chamber, then supplied to the animal via a sideport. Concentrations in the exposure chamber were monitored with a Miran 1A Gas Analyzer. Chamber air flow, temperature, relative humidity, and oxygen were monitored as well. Each animal was exposed individually. The animal was first secured in a sling, and the snout placed in a modified dog anesthesia mask. The snout went through a small hole in a rubber diaphragm to provide a seal. The animal breathed either chamber atmosphere or room air via a valve on the exposure line sideport. The animal breathed the HCFC-123 through a two-way non-rebreathing valve to maintain a unidirectional flow of chemical. The exposures were subcontracted to and conducted at Wil Research Laboratories, Inc. (WIL Labs), Ashland, OH. Details of animal maintenance, the exposure system, and description of the exposures are contained in the appendix which is a report submitted by WIL Labs in fulfillment of the requirements of their contract.

**Blood and Tissue Sampling**

A 5.0 cm over-the-needle teflon catheter was inserted into a saphenous vein. The catheter was attached to a three-way valve so that heparinized saline could be used to flush the catheter. A three-ml glass syringe was used to draw blood samples. Three, approximately 100 $\mu$l samples were placed into preweighed headspace vials and reweighed for analysis of HCFC-123 concentration. For tissue sampling, animals were euthanized by lethal injection. The dead animals were transferred as rapidly as possible to a necropsy suite to harvest tissues for HCFC-123 analysis. The intact heart was removed first, followed by samples of fat (perirenal), liver, and skeletal muscle. For each tissue, three subsamples of approximately 500 mg were weighed and sealed in headspace vials. In general, the entire necropsy procedure was completed in less than 5 minutes.

**Analysis of Blood and Tissue Samples for HCFC-123**

Blood and tissue samples were stored in a -80 $^{0}$C freezer until analysis. Headspace vials containing blood or standards were loaded onto a Tekmar 7050 static headspace sampler for injection onto a Varian 3700 Gas Chromatograph. The gas chromatograph was equipped with a 0.53mm x 25m PoraPlot Q column and an electron capture detector. Tissue samples were first digested with sodium hydroxide solution to release the HCFC-123 into the headspace. The digestion process occurred within the headspace vial. The digested samples were analyzed in the same manner as the blood. Sample headspace HCFC-123 concentrations were calculated from a standard curve.

## SECTION 3

## RESULTS

The blood and tissue (heart, muscle, liver, and fat) concentrations for all exposure scenarios are given in Tables 2 and 3, respectively. Results for exposure durations of 1 to 5 minutes are summarized in Table 4. In animals exposed for 60 minutes (n=4), the maximum venous blood concentrations (mean values) were attained within 30 minutes, with less than a 3% increase over the next 30 minutes (Figure 1 and Table 2). Animals allowed to recover for 30 minutes (n=2), had rapid decreases in the venous blood concentrations within the first 16 minutes with concentrations approaching the limits of detection by 31 minutes postexposure (Figure 2 and Table 2).

Figures 3 and 4 are graphs of the triplicate blood concentrations at the early time points for all animals exposed to 1% and 5% HCFC-123, respectively. The experimental design allowed for the sampling of 8 animals at 1.0 minute during the 1% exposures. Due to problems in sampling, half of the 1.0 minute samples were not available for analysis.

The rise and fall in tissue concentrations paralleled that of blood. Heart, liver, and muscle tissue appeared to take up HCFC-123 much quicker than fat tissue (Table 3). It should be noted that the concentration of chemical in fat, in animals exposed for 60 minutes, was approximately 3 to 5 fold higher than the other tissues as expected. The solubility of HCFC-123 in fat (partition coefficient = 52.9) is approximately 25 times greater than muscle (2.3), liver (1.9), or heart (2.5) tissues. Subsequently, the washout of chemical in fat tissue was much slower than any other tissue. In general, the blood and tissue concentrations of HCFC-123 both increased with exposure time and increasing concentration.

**Table 2. Blood Concentrations in Dogs Exposed by Inhalation to HCFC-123**

| 1% Exposure Concentration | Time of Exposure (Min) | | | | | | | | | | | Animal I.D. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 7.5 | 10 | 15 | 30 | 45 | 60 | |
| Blood Concentrations (mg/L) | 7.6 | 10.0 | 11.0 | 11.7 | 9.5 | 7.6 | 7.2 | 10.3 | 22.7 | 13.8 | 15.7 | 1993 |
| | - | 3.3 | 8.8 | 6.6 | 9.1 | 8.2 | 13.6 | 26.1 | 31.3 | 36.1 | 34.5 | 1994 |
| | 4.4 | 4.4 | 3.8 | 4.9 | 5.4 | 10.2 | 12.0 | 24.7 | 33.2 | 33.5 | 33.6 | 1992 |
| | 4.1 | 10.6 | 16.5 | 18.2 | 18.4 | 17.2 | 14.3 | 21.1 | 22.9 | 27.3 | 29.1 | 1995 |
| | - | 0.7 | 1.5 | 2.3 | 2.7 | - | - | - | - | - | - | 1975 |
| | - | 3.3 | 6.0 | 7.3 | - | - | - | - | - | - | - | 1986 |
| | 4.7 | - | - | - | - | - | - | - | - | - | - | 1999 |
| | - | 3.8 | - | - | - | - | - | - | - | - | - | 1974 |
| Mean | 5.2 | 5.2 | 4.6 | 8.5 | 9.0 | 10.8 | 11.8 | 20.6 | 27.5 | 27.8 | 28.2 | |

| 5% Exposure Concentration | Time of Exposure (Min) | | | | | Animal I.D. |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| Blood Concentrations (mg/L) | 5.8 | 13.0 | 21.5 | 40.5 | 84.4 | 1997 |
| | 7.8 | 109.9 | 131.2 | 125.2 | 145.4 | 1983 |
| | 43.2 | - | - | - | - | 1979 |
| | 28.9 | - | - | - | - | 1990 |
| Mean | 21.4 | 61.4 | 76.3 | 82.8 | 114.9 | |

| 1% Exposure Concentration | Time Post 60 Minute 1% Exposure (Min) | | | | | Animal I.D. |
|---|---|---|---|---|---|---|
| | 1 | 3 | 6 | 16 | 31 | |
| Blood Concentrations (mg/L) | 14.0 | 9.4 | 7.6 | 5.3 | 4.0 | 1993 |
| | 30.6 | 27.8 | 25.1 | 6.7 | - | 1994 |
| Mean | 22.3 | 18.6 | 16.3 | 6.0 | 4.0 | |

**Table 3. Tissue Concentrations in Dogs Exposed by Inhalation to HCFC-123**

| Exposure Concentration Exposure Time | Tissue Concentrations (mg/L) | | | | Animal I.D |
|---|---|---|---|---|---|
| | Heart | Muscle | Liver | Fat | |
| One Percent One Minute | 14.7 | 13.8 | 12.9 | 2.1 | 1999 |
| | 6.7 | 7.0 | 5.1 | 0.6 | 1974 |
| One Percent Five Minutes | 15.8 | 5.5 | 14.6 | 15.9 | 1975 |
| | 18.6 | 7.2 | 19.5 | 13.9 | 1986 |
| One Percent Sixty Minutes | 39.4 | 34.7 | 51.1 | 199.1 | 1992 |
| | 37.9 | 66.6 | 46.0 | 182.2 | 1995 |
| One Percent Sixty Minutes[1] | 2.5 | 5.3 | 2.2 | 118.5 | 1993 |
| | 2.6 | 10.2 | 3.6 | 195.3 | 1994 |
| Five Percent One Minute | 107.4 | 24.9 | 75.8 | 3.9 | 1979 |
| | 94.8 | 29.1 | 48.2 | 9.7 | 1990 |
| Five Percent Five Minutes | 141.0 | 39.5 | 81.8 | 78.3 | 1997 |
| | 179.8 | 36.4 | 174.9 | 46.2 | 1983 |

[1]Samples collected thirty minutes post exposure.

**Table 4. Blood and Tissue Concentrations[1] in Dogs Exposed by Inhalation to 1% or 5% HCFC-123. Number of dogs was two unless noted otherwise.**

| Sample | One Percent | | Five Percent | |
|---|---|---|---|---|
| | 1 minute | 5 minutes | 1 minute | 5 minutes |
| Blood | 5.2(4.1-7.6)[2] | 9.0(2.7-18.4)[3] | 21.4(5.8-43.2)[2] | 114.9(84.4-145.4) |
| Heart | 10.7(6.7-14.7) | 17.2(15.8-18.6) | 101.1(94.9-107.4) | 160.4(141.0-179.8) |
| Muscle | 10.4(7.0-13.8) | 6.4(5.5-7.2) | 27.0(24.9-29.1) | 38.0(36.4-39.5) |
| Liver | 9.0(5.1-12.9) | 16.8(14.4-19.2) | 62.0(48.2-75.8) | 128.3(81.8-174.9) |
| Fat | 1.36(0.6-2.1) | 14.9(13.9-15.9) | 6.8(3.9-9.7) | 62.3(46.2-78.3) |

[1] concentrations in mg/L expressed as mean (range)
[2] number of dogs was 4
[3] number of dogs was 5

## SECTION 4

## DISCUSSION

Potential occupational military exposure to HCFC-123 as a fire extinguishant is expected to be brief with low probability of repeated exposures. The selection of a 1-minute exposure duration represents a "typical" emergency exposure scenario outdoors or indoors where the area can be evacuated after use of a fire extinguisher. A biological endpoint considered for establishing an upper concentration for safe use of a fire extinguishant is cardiac sensitization.

As expected, blood and tissue concentrations both increased with exposure time and increasing concentration. Blood and tissue concentrations after a 5-minute exposure to 1% HCFC-123 (the NOAEL) can be assumed to be the HCFC-123 concentrations at which there would be no cardiac arrhythmias. Blood concentrations and heart tissue concentrations are considered to be most relevant to the endpoint of cardiac sensitization. Table 4 shows that Haber's Law is not appropriate for estimating pharmacokinetic levels, because the blood concentration at 5% HCFC-123 for 1 minute was approximately 2.5 times greater than 1% for 5 minutes, and the heart tissue concentration was approximately 6 times greater in the higher concentration, shorter duration exposure than in the lower concentration, longer duration exposure.

Blood concentrations measured in dogs after exposure to 1% HCFC-123 for 5 minutes (the NOAEL) averaged 9.0 (range 2.7-18.4) mg/L. If we assume that sensitization will not occur if this concentration is not exceeded (Beck et al., 1973), we can extrapolate the 5.2 mg/L blood concentration measured at 1% exposure for 1 minute to an exposure of 1.9% for 1 minute to give a 9.0 mg/L blood concentration. Extrapolating the heart concentrations in the same way gives an exposure of 1.3% for 1 minute to give a 17.2 mg/L heart concentration. However, ideally the pharmacokinetic data collected from the dog studies described above should be used to validate a physiologically based pharmacokinetic model which can be used to predict given blood or heart concentrations from a given exposure scenario.

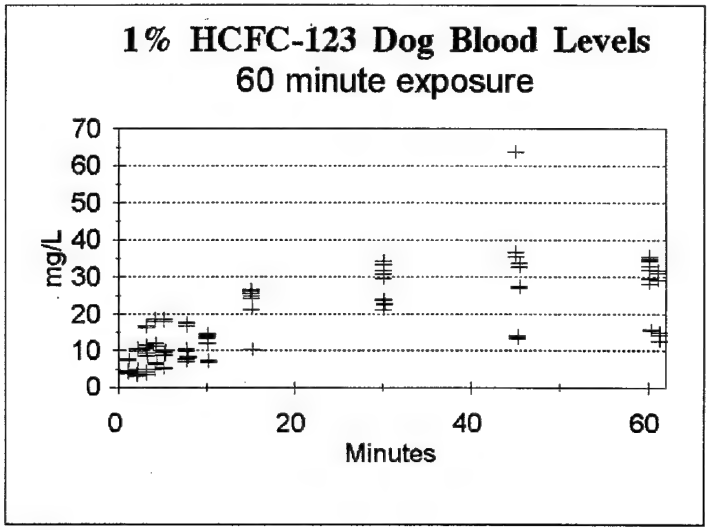


**Figure 1**

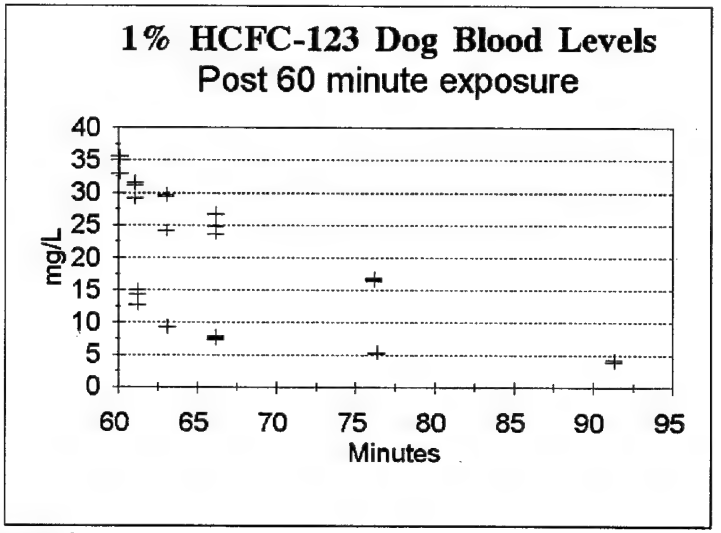


**Figure 2**



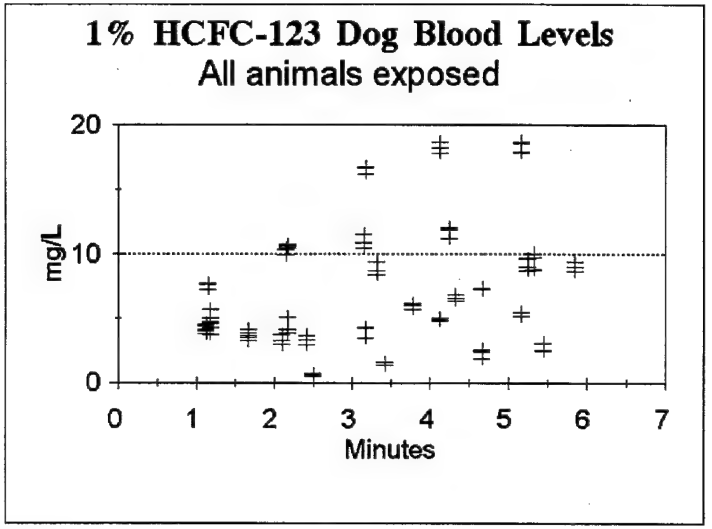


**Figure 3**

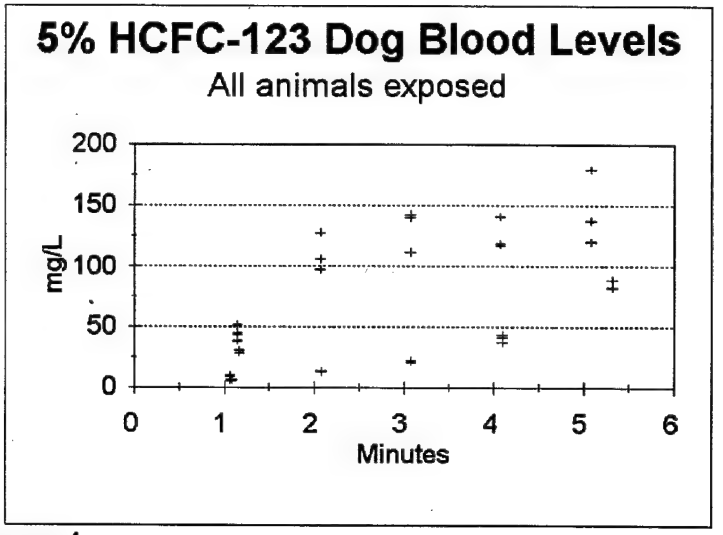


**Figure 4**

## SECTION 5

## REFERENCES

**Beck, P.S., D.G. Clark, and D.J. Tinston.** 1973. The pharmacologic actions of bromochlorodifluoromethane (BCF). *Toxicol. Appl. Pharmacol.* **24**:20-29.

**Haber, F.** 1924. Zur Geschichte des Gaskrieges. In Fuenf Vortraege aus den Jahren 1920-1923, pp. 75-92. Verlag Julius Springer, Berlin.

**Jarabek, A.M., J.W. Fisher, R. Rubenstein, J.C. Lipscomb, R.J. Williams, A. Vinegar, and J.N. McDougal.** 1994. Mechanistic insights aid the search for CFC substitutes: Risk assessment of HCFC-123 as an example. *Risk Analysis* **14**:231-250.

**Trochimowicz, H.J. and L.S. Mullin.** 1973. Cardiac sensitization potential ($EC_{50}$) of trifluorodichloroethane. Haskell Laboratory Report No. 132-73.

**APPENDIX**

**Final Report**

**From**

**Wil Research Laboratories, Inc.**

**Ashland, Ohio**

Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

## COMPLIANCE STATEMENT

As per agreement with the Sponsor, this study was not conducted in strict compliance with the U.S. EPA Good Laboratory Practice Regulations (40 CFR, Part 792). This lack of compliance applies to the involvement of the WIL Research Laboratories, Inc. Quality Assurance Unit, which did not perform critical phase, raw data or final report audits. Otherwise, all reasonable efforts were made to adhere to the protocol, all relevant SOPs, and the spirit and intent of the GLPs.

Study Director: _____ 1 July 94
Christopher P. Chengelis, Ph.D., D.A.B.T. — Date
Associate Director of Toxicology

Sponsor: _____ 7/5/94
Sponsor Agent — Date

Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

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Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

## I. SUMMARY:

The objective of this study was to provide data to validate a physiologically-based model of the pharmacokinetic behavior of the test article (HCFC-123) in the male beagle dog. Two dogs per time-point were exposed to HCFC-123 at 1% for 1, 5 and 60 minutes, and a final group was exposed for 60 minutes, but received a 30 minute recovery (or wash-out) period. Separate groups of dogs were exposed for 1 and 5 minutes to 5% HCFC-123. Exposure was nose-only via a specially adapted canine anesthesia mask equipped with a two-way, non-rebreathing valve. The exposure system was specially designed to permit instantaneous exposure of the dogs to the target concentrations, and permit the drawing of exhaled breath samples and collection of expired air. During exposure, concentrations were carefully monitored, blood and expired air samples were collected for HCFC-123 analysis, and each dog's minute volume of respiration was determined. At the end of the exposure periods (or the appropriate recovery periods), animals were euthanized and selected tissues (heart, liver, kidney, fat, duodenum, and skeletal muscle) collected for HCFC-123 analysis.

At the Sponsor's request, an additional experiment was performed. Two dogs were individually exposed for 60 minutes to 1% HCFC-123 in a 1.5 $m^3$ whole body inhalation chamber. Blood samples (starting at $T_{99}$) were taken at 1, 2, 4, 5, 10, 15, 30, 45 and 60 minutes of exposure and transferred to the Sponsor. After cessation of exposure, dogs were returned to the stock dog colony.

The male dogs assigned to study weighed between 8 and 16 kilograms and were in apparent good health. Pretreatment ECGs were normal. Exposure and data collection proceeded without the occurrence of a major confounding incident. Two types of incidents were noted, however, which may affect data interpretation. First, dogs became agitated during exposure to the test article. This behavior may have resulted in more rapid and shallow breathing. Secondly, some of the blood samples drawn, using standard glass syringes as specified by the protocol, were obviously contaminated with air.

Mean atmospheric concentrations of HCFC-123 for both experiments were within 4% of the target concentrations. Respiratory minute volumes (first experiment) ranged from 1.6 to 5.2 liters per minute.

The responsibility of WIL Research Laboratories, Inc. was to expose the animals, assist as required in the collection of the samples, and prepare an appropriate report on the methods applied.

Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

## II. OBJECTIVE

The objective of this study was to provide data to validate a physiologically-based pharmacokinetic model of the behavior of the test article (HCFC-123) in the male beagle dog. Dogs were exposed (using nose-only procedures) to HCFC-123 at two concentrations for varied durations. During exposure, blood samples and samples of expired air were taken for HCFC-123 analysis. In addition, expired air was collected for the determination of respiratory minute volumes. At the end of the exposure or recovery periods, animals were euthanized and selected tissues collected for HCFC-123 analysis.

At the Sponsor's request, additional experimentation was performed after the completion of the aforementioned study. In this experiment, two dogs were exposed individually to a single concentration of HCFC-123 in a whole body chamber. During exposure, blood samples were collected for HCFC-123 analysis.

The responsibility of WIL Research Laboratories, Inc. was to expose the animals, assist as required in the collection of the samples, and prepare an appropriate report on the methods applied. The Sponsor assumed responsibility for the analysis of all samples, and the interpretation and reporting of the data. This document constitutes the final report on the aspects of this project for which WIL Research Laboratories, Inc. was responsible.

## III. STUDY DESIGN

### A. Probe Experiment

A preliminary (probe) experiment was conducted on November 3, 1993 to test equipment and procedures used for the main (first) experiment described below. Two male beagle dogs were selected from available stock and one each exposed to 1 and 5% (v/v) HCFC-123. This experiment will not be discussed in further detail.

### B. Main (First) Experiment

The study design is described in greater detail in the study protocol (Appendix D). Twelve dogs were assigned to this experiment; eight (group 1) were exposed to 1% (v/v) HCFC-123, and four (group 2) were exposed to 5% HCFC-123, by nose-only techniques. Exposure periods were 1, 5, 60 minutes and 60 minutes plus a 30-minute recovery period for group 1, while exposure periods were 1 and 5 minutes for group 2. There were two dogs per exposure period per group. All exposures were initiated and completed on November 24, 1993.

During exposure, blood samples were collected (as applicable) at 0 (pre-exposure), 1, 2, 3, 4, 5, 7.5, 15, 30, 45 and 60 minutes. During the recovery periods, blood samples were collected at 2, 5, 15 and 30 minutes post-exposure. During the exposure periods, samples of expired air were taken for HCFC-123 determinations at the same times as blood sample collections. In addition, 1- to 5-minute samples of expired air were collected, volumes were determined and minute volumes were calculated.

Dogs were terminated by lethal injection (Socumb® Euthanasia Solution; a barbiturate and potassium chloride mixture) immediately after the final blood samples were taken, and selected tissues were sampled as quickly as possible for HCFC-123 analysis. All samples for HCFC-123 analysis were transferred to the Sponsor, who transported them to their facilities for analysis.

### C. Follow-up (Second) Experiment

Additional experimentation was conducted (April 5, 1994) at the request of the Sponsor. In this study, two male dogs were individually exposed to 1% HCFC-123 for 60 minutes in a whole body chamber. Blood samples were collected at 0 (pre-

exposure) 1, 2, 4, 5, 10, 15, 30, 45 and 60 minutes. Samples were transferred to Sponsor representatives. Dogs were returned the stock colony.

## IV. EXPOSURE SCHEDULE AND TREATMENT REGIMEN

### A. Main (First) Experiment (November 19, 1993)

Animals were exposed sequentially to HCFC-123 as shown below:

| Animal Number | Time of Exposure (minutes) | Exposure Concentration (% v/v) |
|---|---|---|
| 1999 | 1 | 1 |
| 1995 | 60 | 1 |
| 1975 | 5 | 1 |
| 1994 | 60 + 30[a] | 1 |
| 1974 | 1 | 1 |
| 1992 | 60 | 1 |
| 1986 | 5 | 1 |
| 1993 | 60 + 30[a] | 1 |
| 1979 | 1 | 5 |
| 1997 | 5 | 5 |
| 1990 | 1 | 5 |
| 1983 | 5 | 5 |

[a] = 60 minutes of exposure followed by 30 minutes of recovery.

### B. Follow-Up (Second) Experiment (April 5, 1994)

| Animal Number | Time of Exposure (minutes) | Exposure Concentration (% v/v) |
|---|---|---|
| 2154[b] | 25 | 1% |
| 2276 | 60 | 1% |
| 2242 | 60 | 1% |

[b] = The Protocol Amendment II specified the exposure of two animals. The exposure of this first animal was aborted because of a severe reaction to the test article. This first animal was replaced with animal no. 2242.

## V. MATERIALS AND METHODS

### A. TEST ARTICLE

The test article, HCFC-123 (dichlorotrifluoroethane), was synthesized by the E.I. DuPont de Nemours Company. It was purchased by the Sponsor (ManTech Environmental Technology, Inc.) and shipped to WIL Research Laboratories, Inc., where it was received in a single unpressurized steel drum (gross weight of 50.85 kg and a net weight of 45.41 kg) on October 14, 1993. It was assigned assession number WIL-2535A. A specific manufacturer's lot number was not provided on the label or accompanying documentation.

Data on the stability and purity for the test article were the responsibility of the Sponsor. Samples of neat material were provided to the Sponsor upon receipt of the material and upon termination of the main experiment.

The documentation received with the test article indicated that storage at room temperature was acceptable. However, the material was quite volatile, and to facilitate handling, was stored in a freezer (-20°C).

### B. TEST ATMOSPHERE GENERATION AND MONITORING

The test article was present in the test atmospheres as a gas. The test article was evaporated by heating a reservoir (glass, three-necked, round-bottom flask) in a water bath while air was passed across the surface. Exact test atmosphere concentrations were recorded every time a blood sample was taken. Concentrations were monitored by a Miran 1A Gas Analyzer. Details of the generation and monitoring systems are provided in the appended Inhalation Laboratory Reports (Appendix A for the first experiment and Appendix B for the second experiment). Mass air flow, temperature, relative humidity, and oxygen levels were monitored continuously, and were recorded every five minutes. Results are given in Appendices A and B.

### C. EXPOSURE METHODS (MAIN EXPERIMENT)

Each animal was exposed individually. Each animal was first secured in a sling, and then fitted with a modified dog anesthesia mask. A rubber diaphragm with a small hole was used to provide a seal around the snout. The

test article was first brought to target concentration in a 500-liter (NYU type) inhalation chamber, then supplied to the animal via a sideport. The side port connection was valved such that the animal was breathing either chamber atmosphere or room air. This arrangement made it possible to instantaneously expose the animal to a target concentration; that is, it avoided having to place the animal in a chamber, then initiating test article flow and waiting for the chamber to achieve target concentration. The animal breathed the test atmosphere through a two-way, non-rebreathing valve, to maintain a unidirectional flow of test article, and to prevent the animal from rebreathing the test article which could be at a different (e.g. "nontarget") concentration. The exhalation side of the non-rebreathing valve had an additional valve such that expired air could be directed toward either the exhaust air system or into a Tedlar® bag for determining exhaled air volumes. Valves and connecting tubing were of metal composition (brass, copper, or stainless steel) so that potential interactions of the test article with plastic could be limited. The exhalation side of the system was also equipped with a sampling port, such that air samples could be drawn for test article concentration analysis. More details on the generation and exposure systems are presented in Appendix A.

Immediately prior to the exposure, blank air samples were drawn from room air to determine if there were any leaks in the system such that animals may have been exposed to small amounts of test article prior to the intended exposure.

D. Organization and Treatment Regimen

1. Main (First) Experiment

Individual animals were exposed sequentially as described in Section IV. Blood and expired air samples were collected during the exposure period. Immediately after the final blood samples were drawn, the animals were euthanized by injection of Socumb® and transferred immediately to a separate necropsy room where the target tissues were harvested. Time was carefully monitored by a designated timekeeper. A stopwatch was started upon

initiation of exposure and was stopped when the last tissue sample was taken. The precise time that each critical event occurred was recorded on data collection sheets provided by the Sponsor. These were returned to the Sponsor.

2. Follow-Up (Second) Experiment

The second experiment was of much simpler design than the first. Each dog was secured in a standard canvas sling and placed in a 1.5 $m^3$ stainless steel and glass whole body inhalation chamber (NYU type) of modified glove box design. The test article was generated in the same fashion as in the first experiment. The use of a whole body chamber made the instantaneous exposure of the dogs to the target concentration (1%) impossible, as gas flow could not be initiated until the chamber was sealed. It took approximately 7-12 minutes for the chamber to reach 99% of target concentration ($T_{99}$). After 60 minutes of exposure at the target concentration, the flow of gas was terminated and the animals removed from the chamber once the concentration of HCFC-123 was no longer detectable. The precise time that each critical event occurred was recorded on data collection sheets provided by and returned to the Sponsor. Dogs were returned to the stock dog colony.

# VI. ANIMALS

## A. ANIMAL RECEIPT AND ACCLIMATION

### 1. Main (First) Experiment

Sixteen male beagle dogs were received from LBL Kennels of Reelsville, Indiana, on November 9, 1993. Each animal was identified uniquely with a USDA identification tag affixed to a metal collar chain by the supplier. LBL Kennels is a USDA approved vendor and the animals were accompanied by all legally required paperwork. Animals were to be of approximately one year of age; however, inspection of the animals by WIL Research personnel led to the conclusion that several of the animals were considerably more than one year of age. The vendor made a second trip on November 16, 1993. Ten dogs were returned and ten replacement animals were received as well as documentation as to a birth date. Replacement dogs were inspected by the study director and the staff veterinarian at receipt. All dogs assigned to the study had birth dates between October 30 and November 22, 1992.

According to the documents received from the supplier, all dogs had received the appropriate veterinary care, vaccinations and other medications. To further assure the good health of the dogs, they were further treated (prior to exposure) by WIL Research Laboratories, Inc. veterinary personnel with Nemex® suspension and Primor® tablets.

One dog from the second shipment was found dead on the day after receipt. Post-mortem examination of this dog suggested that the cause of death could have been acute fulminating Parvo virus infection. While this animal was vaccinated, it may have been an animal in which the vaccine was not effective. None of the other animals displayed signs of a similar infection, and the unexpected death of this one animal prior to the start of the dosing period did not affect the quality or integrity of the data collected from the animals assigned to test article exposure.

According to the original protocol, the animals were to receive a two week acclimation period. The protocol was amended so that a one week

acclimation period was acceptable in order to accommodate the animals received in the second shipment while still keeping the project on schedule. Dogs were weighed upon receipt. Also during the acclimation period, they were observed twice daily for changes in appearance and behavior, and were acclimated to the sling and face mask. In the opinion of the study director, the fact that some of the animals had a shorter acclimation period than others had no known impact on the quality of the data and the outcome of the study.

2. Follow-Up (Second) Experiment

Three dogs were selected from the WIL Research stock dog colony. Two were scheduled as per protocol amendment, and one for backup purposes. The animals were male Beagle dogs weighing between 9 and 16 kg. Animal no. 2276 was obtained from Ridglan Farms (Mt. Horeb, WI) and was approximately 6 months old on the day of exposure. Animal no. 2154 was obtained from Harlan Sprague-Dawley (Madison, WI) and was approximately nine months old when exposed. Animal no. 2242 was also obtained from Harlan Sprague-Dawley, and was approximately 10 months old at exposure. They were acclimated to sling and exposure conditions during the week prior to test article administration.

B. ANIMAL HOUSING

The animals were housed individually in stainless steel cages which were cleaned daily during the acclimation period and throughout the study. The animals were maintained in these cages except during the exposure and exercising periods. The animals were exercised in accordance with the Final Rules of the Animal Welfare Act Regulations (9 CFR, Part 3). Animals were maintained in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals." The animal facilities at WIL Research Laboratories, Inc. are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

C. DIET, DRINKING, WATER AND MAINTENANCE

Approximately 400 g of Purina® Certified Canine Chow® #5007, was offered

daily. The diet utilized at WIL Research Laboratories, Inc., is a certified feed with appropriate analyses for potential contaminants performed and provided by the manufacturer. The feed and drinking water, delivered by an automatic watering system, were provided *ad libitum* throughout the study period, except during the exposure and exercising periods. Municipal water supplying the facility was sampled for contaminants according to Standard Operating Procedures. The results of the diet and water analyses are maintained at WIL Research Laboratories, Inc. No contaminants were present in animal feed or water at concentrations expected to interfere with the objectives of this study.

D. ENVIRONMENTAL CONDITIONS

Except during exposure, all animals were housed throughout the acclimation period and during the study in environmentally controlled rooms. Controls were set to maintain a temperature of 72° ± 3°F and a relative humidity of approximately 30-70%. Room temperature and relative humidity were recorded daily. The actual temperature ranged from 68°F to 72°F and relative humidity ranged from 46% to 76%. Occasional variations in room temperature and relative humidity during the study period did not apparently affect the outcome of this study. Light timers were set to provide a 12-hour light/12-hour dark photoperiod.

## VII. PARAMETERS EVALUATED

### A. Clinical Observations and Survival

Physical examinations were performed the day prior to exposure. Unusual behavior, if any, noted during the exposure periods was recorded on the data collection sheets supplied by the Sponsor.

### B. Body Weights (Main Experiment)

Body weights were determined as described in acclimation (VI.A.1). In addition, body weights were recorded on the Sponsor-supplied data forms immediately prior to exposure. These forms were retained by the Sponsor.

### C. Electrocardiographic Examination (Main Experiment)

Electrocardiograms were continuously recorded with a Cambridge 3038/2 electrocardiograph during the exposure of the two probe animals. These tracings were supplied to the Sponsor, and will not be further discussed here.

Electrocardiograms were recorded using the Vetronics System 3 on the main study dogs once during the acclimation period.

### D. Blood and Expired Breath Sampling

#### 1. Main (First) Experiment

Blood samples were drawn for the purposes of determining test article concentrations. A 5.0-cm (2") over-the-needle Teflon® catheter (Baxter Healthcare Corporation) was first primed with heparinized saline, then inserted into a saphenous vein. The needle was withdrawn, and the catheter was left in place and connected to a three-way, stainless steel valve with a syringe of heparinized saline attached. This was used to keep the catheter flushed between blood samples. At the sampling times, blood was drawn into the three-way valve using the heparin containing syringe then the sample was drawn using a 3-ml glass syringe. The samples were transferred to Sponsor representatives for further processing. During exposure, blood samples were collected (as applicable) prior to exposure and at 1, 2, 3, 4, 5, 7.5, 15, 30, 45 and 60 minutes during exposure. During the recovery periods, blood samples were collected at 2, 5, 15 and 30 minutes post-exposure.

During the exposure periods, samples of expired air (10 cc) were obtained with a gas-tight syringe at the same time as blood samples. These were taken at the exhalation side sampling port as described in Section V.B above and in greater detail in Appendix A. These samples were immediately transferred to the Sponsor for subsampling and preparation for transfer to their laboratory.

2. Follow-Up (Second) Experiment

After the animal was secured in a sling, a 5-cm Teflon® catheter (as described above) was placed in each cephalic vein, fitted with Jelco™ injection caps and flushed with heparinized saline. The Sponsor provided a specially constructed stainless steel tube and three-way stopcock apparatus. This was placed through a small hole in the chamber wall and inserted into the gel cap. Blood was then drawn and the tube flushed as described above. Both forelimbs were prepared for backup purposes. Blood samples were collected at 0 (pre-exposure) 1, 2, 4, 5, 10, 15, 30, 45 and 60 minutes starting when the chamber concentration reached $T_{99}$.

E. Tissue Collection (First Experiment Only)

Animals were euthanized by lethal injection (Socumb® Euthanasia Solution, a mixture containing a barbiturate and potassium chloride) via the catheter immediately after the last blood samples were taken. The carcasses were then transferred as rapidly as possible to a necropsy suite for further processing. The necropsy was conducted only to harvest tissues for test article analysis. Identification of potential test article related anatomic changes was not an objective, and gross observations were not recorded. The intact heart was first removed as rapidly as possible. Samples of fat (perirenal), duodenum, liver and skeletal muscle (quadriceps) were then taken. One kidney was removed intact. The Sponsor personnel sub-sampled and weighed the tissues in glass vials, and prepared them for transfer to their laboratory for test article analysis. The times for all samples were taken and transferred were recorded relative to the time exposure was initiated. The times that the glass vials were sealed were recorded on the forms

provided by the Sponsor. In general, the entire necropsy procedure was completed in less than 10 minutes.

F. Respiratory Minute Volumes (First Experiment Only)

Expired air samples were collected for defined times. For the 1- and 5-minute exposures, expired air was collected for the entire exposure period. For the 60-minute exposures, air was collected for five minute periods starting at 10, 25, 40 and 55 minutes after the initiation of exposure. Exhaled volumes were determined by emptying the collection bags through a calibrated flow meter. Details on the flow meter and the calibration process are presented in Appendix A. Minute volumes were calculated by dividing the volumes by time length of the collection period.

G. DATA RETENTION

Upon acceptance of the final report, all raw data were transferred to the Sponsor.

## VII. RESULTS AND DISCUSSION

### A. Main (First) Experiment

Dogs assigned to study weighed between 8.7 and 13.1 kg and were in apparent good health (Table 1). Pretreatment ECGs were normal. Exposure and data collection proceeded without the occurrence of a major incident; however, several dogs became agitated and hyperactive during exposure and at times had to be securely held in position. This behavior may have resulted in more rapid and shallow breathing which may have affected the respiratory uptake of the test article. Agitated behavior was recorded individually on the data forms provided, which were returned to the Sponsor.

Precise atmospheric concentrations of HCFC-123 at blood sampling intervals are summarized in Table 1 of Appendix A. In general, all readings were within 4% of the target concentrations.

Respiratory minute volumes are summarized in Table 3 of Appendix A. Minute volumes ranged from 1.6 to 5.2 liters.

### B. Follow-Up (Second) Experiment

Dogs assigned weighed 8 to 11 kg. All dogs, to varying degrees, became aggressive and hyperactive during exposure. In one instance, it became necessary to terminate exposure, and repeat the exposure with a replacement dog. Similar behavior was noted in the first experiment, but the animals were easier to control when they were not in an inhalation chamber.

Atmospheric concentrations of HCFC-123 during the exposure period (after the attainment of $T_{99}$) are summarized in Table 1 of Appendix B. In general, all readings were within 3% of the target concentration.

In both experiments, 2-ml glass syringes, as specified in the protocol, were used to draw the blood samples. On some occasions (documents returned to Sponsor) the blood samples took on a frothy appearance while being drawn, and were obviously contaminated with air. It is recommended that gas-tight glass syringes be used for any similar future work.

Christopher P. Chengelis, Ph.D., D.A.B.T.
Study Director

7/1/94
Date

## VIII. STUDY PERSONNEL AND REPORT SUBMISSION:

Reviewed By:

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Prepared, Approved and Submitted By:

7/1/94
Christopher P. Chengelis, Ph.D., D.A.B.T. — Date
Associate Director of Toxicology

Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

Table 1

**TABLE 1**

ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS
BY INHALATION ANIMAL BODY WEIGHT/CLINICAL OBSERVATIONS
Main (First) Experiment
(Day Prior to Exposure)

| Animal | Body Weight (kg) | Clinical Observations |
|---|---|---|
| 1999 | 13.088 | No significant clinical observations |
| 1995 | 9.328 | No significant clinical observations |
| 1974 | 12.047 | No significant clinical observations |
| 1994 | 11.228 | No significant clinical observations |
| 1975 | 9.113 | No significant clinical observations |
| 1990 | 8.485 | Lacrimation left eye |
| 1992 | 10.155 | No significant clinical observations |
| 1993 | 9.003 | No significant clinical observations |
| 1979 | 11.128 | No significant clinical observations |
| 1997 | 9.552 | Lacrimation right eye, scabbing right ear<br>Scabbing right ear |
| 1986 | 10.708 | No significant clinical observations |
| 1983 | 8.709 | No significant clinical observations |

Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

# APPENDIX A

Test Atmosphere Generation and Validation and
Environmental Conditions During Exposure - Main (First) Experiment

Acute Pharmacokinetic Study of
HCFC-123 in Dogs by Inhalation

## APPENDIX A

### Table of Contents

Acute Pharmacokinetic Study of
HCFC-123 in Dogs by Inhalation

## APPENDIX A

## Index of Tables

## I. ANALYTICAL METHODS

### A. Summary

The test article, HCFC-123, was analyzed by infrared spectrometry with a Foxboro Miran 1A Gas Analyzer. The atmospheres (10,000 and 50,000 ppm ) were generated in a 0.5 $m^3$ (500-liter) whole body inhalation chamber modified for nose-only exposure to dogs.

### B. Instrumentation and Methods

Infrared Spectrometry

| | |
|---|---|
| Instrument: | Foxoboro Miran 1A Gas Analyzer with a 20 m cell |
| Windows: | NaCl |
| Pathlength: | 0.75 m |
| Wavelength: | 12.7 $\mu$m |
| Slit Width: | 1 mm |
| Cell Volume: | 5.6 liters (5.64 liters with calibration loop) |

### C. Miran 1A Calibration

#### 1. Methods

Standards of HCFC-123 in air were prepared using the closed-loop calibration system supplied with the Miran 1A. The volume of the calibration system was 5.64 liters. For this volume, the appropriate amounts of HCFC-123 were determined by calculation, and then injected into the calibration system using a Pressure-Lok® Series A-2 1.0-ml gas syringe which was maintained on ice to prevent excessive volatization of the test article. The following formulae were used to calculate standard concentrations for calibration:

$$\frac{\text{ml HCFC-123 (liquid) x Specific Gravity (g/ml)}}{\text{Molecular Weight (g/mol)}} = \text{moles HCFC-123}$$

$$\text{mol HCFC-123 x 24.45 L/mol x 1,000 ml/l} = \text{ml HCFC-123 (gas)}$$

$$\frac{\text{ml HCFC-123 (gas)}}{\text{Loop Volume}} \times 10^6 = \text{concentration of standard (ppm)}$$

where:

| | |
|---|---|
| Specific Gravity = | 1.46 g/ml |
| Molecular Weight = | 152.93 g/mol |
| Calibrator Volume = | 5640 ml |
| ppm = | Parts per million |

Four standards were prepared in triplicate on two occasions during method development and once on the day prior to exposure. The average absorption (X) was plotted against concentration (Y) to construct a calibration curve, using the linear regression routine in a Hewlett-Packard calculator model HP-20S. The concentration of each atmosphere sample was calculated from this curve.

2. Results

Mean calibration responses were consistent over the test period. Calibration data for method development and animal exposure are presented in Table 2.

## II. EXPOSURE METHODS

### A. Inhalation Exposure System Description

The test atmospheres were generated in a 0.5 $m^3$ (500-liter) glass and stainless steel whole body inhalation chamber (NYU Type). The chamber was operated at approximately 10 air changes per hour (approximately 83 liters per minute for a 500-liter chamber). A HEPA filter and an activated carbon cartridge filter were used to treat room temperature air before it entered the chamber. Mass airflow through the chamber was determined by measuring the pressure drop across an orifice plate as monitored by a Dwyer Magnehelic® Indicating Transmitter pressure gauge. A Kurz mass airflow meter was utilized as a fail-safe device. Negative pressure within the chamber was monitored by a Dwyer Magnehelic® Indicating Transmitter. Treatment of exhaust air consisted of drawing the air through an activated carbon bed, a HEPA filter and a water-spray fume scrubber. A Hy-Cal Engineering sensor and transmitter were used to monitor temperature and relative humidity. Oxygen content was measured by an MSA Remote Sampling system. The test atmosphere was continuously drawn to the exposure apparatus from the exposure chamber with a purge flow of approximately 1 lpm. Figure I is a diagram of the exposure system.

Temperature, relative humidity, oxygen content, mass airflow and negative pressure within the chamber were continually monitored and recorded every 30 minutes through the use of Labtech Notebook Data acquisition software, a Packard Bell PB286 computer and a Brother M-1824L printer.

### B. Method of Test Atmosphere Generation

The HCFC-123 atmospheres were generated at concentrations of 10,000 and 50,000 ppm by passing a flow of dry, compressed air across the surface of the liquid test material contained in a 3000-ml three-necked flask which was partially submerged in a water bath. Water bath temperatures of approximately 26°C and 40°C were required at the 1% and 5% concentrations, respectively, to maintain the test material temperature at approximately 20°C (as indicated by a mercury thermometer placed in the liquid test material). Airflows of approximately 3 lpm

and 9 lpm (as measured by a Sierra Instruments Top=Trak™ Digital Flowmeter) were used to deliver the test material to the chamber via 1/4" O.D. stainless steel lines.

## III. EXPOSURE PERIOD CONDITIONS

### A. General Conditions of Exposure

#### 1. Exposure Duration

The length of exposure to test article atmospheres varied from 1 minute to 60 minutes. In addition, two animals were exposed for 60 minutes with a 30-minute recovery period during which the animal remained in the exposure apparatus, but breathed room air only.

#### 2. Animal Placement

The animals were exposed using a modified nose-only apparatus attached to the whole body inhalation chamber (see Figure I).

### B. Inhalation Chamber Environmental Data

#### 1. Methods

Chamber temperature, relative humidity, oxygen content, negative pressure and mass airflow were monitored continually during exposure using the Labtech Notebook Data Acquisition System. Values for these parameters were recorded every thirty minutes.

#### 2. Results

Chamber temperature, relative humidity, oxygen content, negative pressure and mass airflow means are presented in Table 4.

### C. Test Atmosphere Characterization

#### 1. Analyzed Concentrations

##### a. Methods

Test article concentration was monitored continuously by drawing a portion of the test atmosphere through 1/4" O.D. stainless steel tubing to the Miran 1A Gas Analyzer and returning the sample to the generation chamber to be exhausted. The analyzer's pump was used to draw the test atmosphere samples. Test atmosphere concentration was determined by reading and recording the infrared absorbance value at a given time, and entering this value into the linear regression routine to determine the concentration of the test atmosphere in parts per million (ppm).

b. Results

A test atmosphere concentration reading was generally recorded at each blood collection time point. All concentrations were within ± 9% of the target. Individual concentration data are presented in Table 1.

2. Expired Air Volume Calculation

a. Methods

Expired air was collected for specific time intervals at designated time points in a 30-liter Tedlar® bag connected to the nose-only exposure setup. Upon completion of the sampling period, the three way valve was closed to the bag and the expired breath no longer collected. The Tedlar® bag was then evacuated, using a Sierra Instruments Top=Trak™ 0-10 slpm Digital Flowmeter to monitor flow rate (lpm) and a stopwatch to measure time in order to ascertain the volume of expired air.

During preliminary experiments, a procedure was developed which allowed for the calculation of a correction factor (which was concentration-dependent) for the determination of expired air volumes with the digital flowmeter mentioned previously (which was calibrated to air only). To determine the effect of HCFC-123 on the measured flow rate, a 30-liter Tedlar® bag was filled to a known volume [calculated by metering air at a known rate (slpm) for a given duration (minutes)] with air, then a known volume of liquid test material was injected into the bag through the septum port. The bag was then evacuated at a set rate, the time recorded, and the values compared. On performing a linear regression on concentration (X) vs. % difference (Y), the correlation coefficient (r) was calculated to be 0.9995, indicating that there was linear relationship. The slope of the line was calculated to be 0.000421, and the y-intercept was 2.35%. However, in this study, the exact concentration of HCFC-123 in the Tedlar® bag was not known with certainty, because of the amount deposited in the dog. Thus, the chamber concentrations were used to estimate total volumes and minute volumes.

To calculate the estimated total expired air volumes and minute volumes collected during exposure, the following formulae were used:

Correction factor = 0.000421 x measured concentration (ppm) + 2.35%

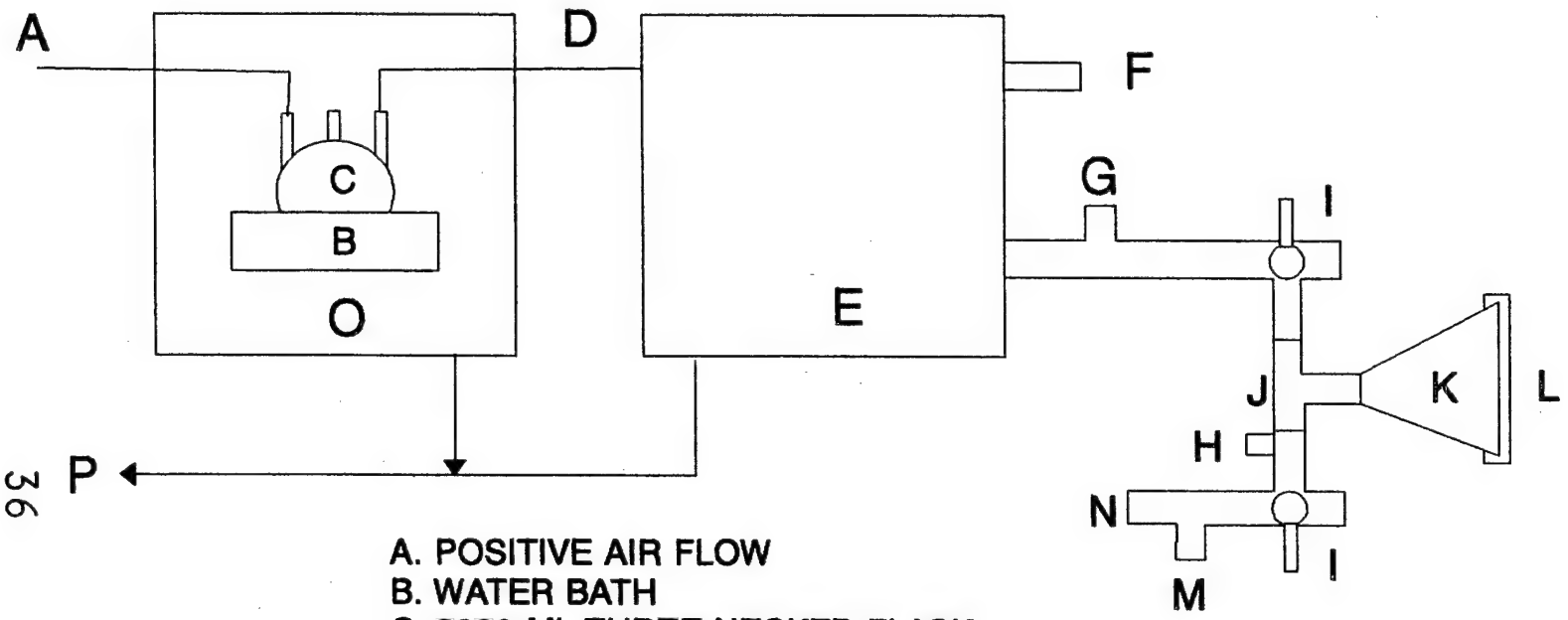
Estimated Volume = [measured volume (1) x 100] / [100 + correction factor]

Minute Volume = Estimated volume (l) / Length of collection (min)

b. Results

The actual estimated total and minute volumes for each dog for each collection time period are presented in Table 3.

# Figure I. Dog Nose-Only Exposure System Setup




A. POSITIVE AIR FLOW
B. WATER BATH
C. 3000 ML THREE NECKED FLASK
D. TEST MATERIAL TO CHAMBER
E. EXPOSURE CHAMBER
F. MIRAN 1A SAMPLING PORT
G. PURGE FLOW
H. EXPIRED AIR SAMPLE PORT
I. THREE WAY VALVE
J. NON-REBREATHING VALVE
K. EXPOSURE MASK
L. RUBBER DIAPHRAM
M. TO TEDLAR BAG
N. EVACUATION PORT
O. GENERATOR ISOLATION CHAMBER
P. TO SYSTEM EXHAUST

APPENDIX A (TABLE 1)

PROJECT NO.:WIL-227001 ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION PAGE 1

TEST ATMOSPHERE CONCENTRATION ANALYSIS RESULTS

TARGET: 1% (10,000 PPM) LENGTH OF EXPOSURE: 1 MINUTE

| ANIMAL NUMBER: | 1999 | | 1974 | |
|---|---|---|---|---|
| | TIME (HH:MM) | CONCENTRATION (PPM) | TIME (HH:MM) | CONCENTRATION (PPM) |
| | 00:00 | 9932 | 00:00 | 10,129 |
| | 00:01 | 9997 | 00:01 | 10,129 |
| MEAN : | | 9965 | | 10,129 |
| S.D. : | | 46.0 | | 0.0 |
| C.V.(%): | | 0.46 | | 0.00 |
| N : | | 2 | | 2 |

APPENDIX A (TABLE 1)

PROJECT NO.:WIL-227001 ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION PAGE 2

TEST ATMOSPHERE CONCENTRATION ANALYSIS RESULTS

TARGET: 1% (10,000 PPM) LENGTH OF EXPOSURE: 60 MINUTES

| ANIMAL NUMBER: | 1995 | | 1992 | |
|---|---|---|---|---|
| | TIME (HH:MM) | CONCENTRATION (PPM) | TIME (HH:MM) | CONCENTRATION (PPM) |
| | 00:00 | 10,392 | 00:00 | 10,195 |
| | 00:01 | 10,260 | 00:01 | 9997 |
| | 00:02 | 10,129 | 00:02 | 9734 |
| | 00:03 | 9997 | 00:03 | 9603 |
| | 00:05 | 9997 | 00:04 | 9471 |
| | 00:06 | 10,195 | 00:05 | 9471 |
| | 00:07 | 10,392 | 00:08 | 9077 |
| | 00:10 | 10,523 | 00:10 | 9274 |
| | 00:13 | 10,655 | 00:15 | 9800 |
| | 00:18 | 10,129 | 00:30 | 9932 |
| | 00:25 | 9997 | 00:45 | 10,063 |
| | 00:30 | 10,392 | 01:00 | 10,260 |
| | 00:40 | 9997 | | |
| | 00:45 | 10,523 | | |
| | 00:55 | 9866 | | |
| | 01:00 | 10,260 | | |
| MEAN : | | 10,232 | | 9740 |
| S.D. : | | 231.5 | | 370.3 |
| C.V.(%): | | 2.26 | | 3.80 |
| N : | | 16 | | 12 |

APPENDIX A (TABLE 1)

PROJECT NO.:WIL-227001 ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION PAGE 3

TEST ATMOSPHERE CONCENTRATION ANALYSIS RESULTS

TARGET: 1% (10,000 PPM) LENGTH OF EXPOSURE: 5 MINUTES

| ANIMAL NUMBER: | 1975 | | 1986 | |
|---|---|---|---|---|
| | TIME (HH:MM) | CONCENTRATION (PPM) | TIME (HH:MM) | CONCENTRATION (PPM) |
| | 00:00 | 10,392 | 00:00 | 10,195 |
| | 00:01 | 10,260 | 00:01 | 9997 |
| | 00:02 | 10,129 | 00:02 | 9734 |
| | 00:03 | 9997 | 00:03 | 9603 |
| | 00:05 | 9997 | 00:04 | 9471 |
| | | | 00:05 | 9471 |
| MEAN : | | 10,392 | | 9734 |
| S.D. : | | 93.0 | | 117.6 |
| C.V.(%): | | 0.89 | | 1.21 |
| N : | | 5 | | 6 |

PROJECT NO.:WIL-227001

APPENDIX A (TABLE 1)
ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION
TEST ATMOSPHERE CONCENTRATION ANALYSIS RESULTS



TARGET: 1% (10,000 PPM)

LENGTH OF EXPOSURE: 60 MINUTES + 30 MINUTE

| ANIMAL NUMBER: | 1993 | 1994 |
|---|---|---|
| TIME (HH:MM) | CONCENTRATION (PPM) | TIME (HH:MM) | CONCENTRATION (PPM) |
| 00:00 | 10,392 | 00:00 | 10,721 |
| 00:02 | 9997 | 00:01 | 10,655 |
| 00:03 | 9734 | 00:02 | 10,392 |
| 00:05 | 9734 | 00:03 | 10,260 |
| 00:08 | 9603 | 00:04 | 10,129 |
| 00:10 | 9866 | 00:05 | 9997 |
| 00:15 | 10,392 | 00:08 | 9997 |
| 00:20 | 9997 | 00:10 | 10,195 |
| 00:30 | 10,523 | 00:15 | 10,786 |
| 00:45 | 10,786 | 00:30 | 10,786 |
| 01:00 | 10,260 | 00:45 | 10,786 |
| | | 01:00 | 10,523 |
| MEAN : | 10,117 | | 10,436 |
| S.D. : | 378.6 | | 312.6 |
| C.V.(%): | 3.74 | | 3.00 |
| N : | 11 | | 12 |

APPENDIX A (TABLE 1)

PROJECT NO.:WIL-227001 ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION PAGE 5

TEST ATMOSPHERE CONCENTRATION ANALYSIS RESULTS

TARGET: 5% (50,000 PPM) LENGTH OF EXPOSURE: 1 MINUTE

| ANIMAL NUMBER: | 1979 | | 1990 | |
|---|---|---|---|---|
| | TIME (HH:MM) | CONCENTRATION (PPM) | TIME (HH:MM) | CONCENTRATION (PPM) |
| | 00:00 | 50,767 | 00:00 | 49,451 |
| | 00:01 | 50,767 | 00:01 | 49,451 |
| MEAN : | | 50,767 | | 49,451 |
| S.D. : | | 0.0 | | 0.0 |
| C.V.(%): | | 0.00 | | 0.00 |
| N : | | 2 | | 2 |

APPENDIX A (TABLE 1)

PROJECT NO.:WIL-227001 ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION PAGE 6

TEST ATMOSPHERE CONCENTRATION ANALYSIS RESULTS

TARGET: 5% (50,000 PPM) LENGTH OF EXPOSURE: 5 MINUTES

| ANIMAL NUMBER: | 1997 | | 1983 | |
|---|---|---|---|---|
| | TIME (HH:MM) | CONCENTRATION (PPM) | TIME (HH:MM) | CONCENTRATION (PPM) |
| | 00:00 | 50,372 | 00:00 | 50,767 |
| | 00:01 | 50,109 | 00:01 | 50,767 |
| | 00:02 | 50,109 | 00:02 | 49,846 |
| | 00:03 | 49,714 | 00:03 | 49,714 |
| | 00:04 | 49,714 | 00:04 | 50,109 |
| | 00:05 | 50,109 | 00:05 | 50,767 |
| MEAN : | | 50,021 | | 50,328 |
| S.D. : | | 258.8 | | 497.1 |
| C.V.(%): | | 0.52 | | 0.99 |
| N : | | 6 | | 6 |

PROJECT NO.:WIL-227001

TABLE 2
ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION
MIRAN 1A CALIBRATION DATA

PATHLENGTH = 0.75 METERS SLIT = 1 mm WAVELENGTH = 12.7 MICRONS

| CALIBRATION DATE | STANDARD CONCENTRATIONS | ABSORBANCE (a) | R VALUE |
|---|---|---|---|
| 10/28/93 | 8,277 PPM | 0.094 | |
| | 12,416 PPM | 0.134 | 0.9996 |
| | 41,386 PPM | 0.367 | |
| | 62,079 PPM | 0.520 | |
| 11/03/93 | 8,277 PPM | 0.093 | |
| | 12,416 PPM | 0.133 | 0.9995 |
| | 41,386 PPM | 0.364 | |
| | 62,079 PPM | 0.513 | |
| 11/23/93 | 8,277 PPM | 0.093 | |
| | 12,416 PPM | 0.130 | 0.9997 |
| | 41,386 PPM | 0.355 | |
| | 62,079 PPM | 0.502 | |

(a) VALUES GIVEN IN ABSORBANCE UNITS. EACH IS THE MEAN OF THREE TRIALS.
mm = MILLIMETERS PPM = PARTS PER MILLION

PROJECT NO.:WIL-227001

APPENDIX A (TABLE 3)
ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION
EXPIRED AIR VOLUME CALCULATIONS
TARGET: 1% (10,000 PPM)



| ANIMAL NUMBER | TIME FRAME OF SAMPLE (MINUTES) | MEASURED VOLUME (LITERS) | CONCENTRATION a (PPM) | CORRECTION (%) | ESTIMATED VOLUME (LITER) | MINUTE VOLUME (LPM) |
|---|---|---|---|---|---|---|
| 1999 | 1 | 4.845 | 9965 | -6.54 | 4.55 | 4.55 |
| 1995 | 10-15 | 12.546 | 10589 | -6.81 | 11.75 | 2.35 |
| | 25-35 | 12.393 | 10195 | -6.64 | 11.62 | 2.32 |
| | 40-45 | 11.615 | 10260 | -6.67 | 10.89 | 2.18 |
| | 55-60 | 10.029 | 10063 | -6.59 | 9.41 | 1.88 |
| 1975 | 5 | 9.506 | 10392 | -6.72 | 8.91 | 1.78 |
| 1994 | 10-15 | 9.811 | 10129 | -6.61 | 9.20 | 1.84 |
| | 25-35 | 9.894 | 10523 | -6.78 | 9.26 | 1.85 |
| | 40-45 | 10.863 | 10786 | -6.89 | 10.16 | 2.03 |
| | 55-60 | 12.894 | 10260 | -6.67 | 12.09 | 2.42 |
| | 10-15 | 23.003 | 0 | -2.35 | 22.47 | 4.49 |
| | 25-30 | 19.760 | 0 | -2.35 | 19.31 | 3.86 |
| 1974 | 1 | 2.440 | 10129 | -6.61 | 2.29 | 2.29 |
| 1992 | 10-15 | 20.876 | 9537 | -6.36 | 19.63 | 3.93 |
| | 25-35 | 27.272 | 9932 | -6.53 | 25.60 | 5.12 |
| | 40-45 | 24.509 | 10063 | -6.59 | 22.99 | 4.60 |
| | 55-60 | 26.591 | 10260 | -6.67 | 24.93 | 4.99 |
| 1986 | 3-5 | 6.171 | 9734 | -6.45 | 5.80 | 2.90 |
| 1993 | 10-15 | 13.719 | 10491 | -6.77 | 12.85 | 2.57 |
| | 25-35 | 11.410 | 10786 | -6.89 | 10.67 | 2.13 |
| | 40-45 | 13.311 | 10786 | -6.89 | 12.45 | 2.49 |
| | 55-60 | 12.138 | 10523 | -6.78 | 11.37 | 2.27 |
| | 10-15 | 19.737 | 0 | -2.35 | 19.28 | 3.86 |
| | 25-30 | 17.125 | 0 | -2.35 | 16.73 | 3.35 |

a = ESTIMATED TOTAL VLOUMES AND MINUTE VOLUMES BASED ON CHAMBER CONCENTRATIONS WHEN REPIRATORY BAG WAS FILLED.

PROJECT NO.:WIL-227001

APPENDIX A (TABLE 3)
ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION
EXPIRED AIR VOLUME CALCULATIONS
TARGET: 5% (50,000 PPM)



| ANIMAL NUMBER | TIME FRAME OF SAMPLE (MINUTES) | MEASURED VOLUME (LITERS) | CONCENTRATION a (PPM) | CORRECTION (%) | ESTIMATED VOLUME (LITER) | MINUTE VOLUME (LPM) |
|---|---|---|---|---|---|---|
| 1979 | 1 | 3.913 | 50767 | -23.73 | 3.16 | 3.16 |
| 1997 | 5 | 9.860 | 50021 | -23.42 | 7.99 | 1.60 |
| 1990 | 1 | 2.817 | 49451 | -23.18 | 2.29 | 2.29 |
| 1983 | 5 | 17.696 | 50328 | -23.55 | 14.32 | 2.86 |

a = ESTIMATED TOTAL VOLUMES AND MINUTE VOLUMES BASED ON CHAMBER CONCENTRATIONS WHEN REPIRATORY BAG WAS FILLED.

PROJECT NO.:WIL-227001

TABLE 4
ACUTE PHARMACOTOXIC STUDY OF HCFC-123 IN DOGS BY INHALATION
CONDITIONS OF EXPOSURE (MEANS)

| CONDITIONS FOR DOG NOSE-ONLY CHAMBER | | EXPOSURE DATE: 11/24/93 | | |
|---|---|---|---|---|
| TEMPERATURE (DEGREES C) | HUMIDITY (%RH) | OXYGEN CONTENT (%) | MASS AIRFLOW (SLPM) | NEGATIVE PRESSURE (IN. H20) |
| 19.6 | 36 | 20.6 | 89 | 0.49 |

C = CELSIUS RH = RELATIVE HUMIDITY SLPM = STANDARD LITERS PER MINUTE IN. H20 = INCHES OF WATER

Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

# APPENDIX B

Test Atmosphere Generation, Validation and
Environmental Conditions During Exposures - Follow-Up (Second) Experiment

Acute Pharmacokinetic Study of
HCFC-123 in Dogs by Inhalation

## APPENDIX B

### Table of Contents

Acute Pharmacokinetic Study of
HCFC-123 in Dogs by Inhalation

## APPENDIX B

## Index of Tables

## I. ANALYTICAL METHODS

### A. Summary

The test article, HCFC-123, was analyzed by infrared spectrometry with a Foxboro Miran 1A Gas Analyzer. The atmospheres (10,000 ppm) were generated in a 1.5 $m^3$ (1500-liter) whole body inhalation chamber.

### B. Instrumentation and Methods

Infrared Spectrometry

| | |
|---|---|
| Instrument: | Foxoboro Miran 1A Gas Analyzer with a 20 m cell |
| Windows: | NaCl |
| Pathlength: | 0.75 m |
| Wavelength: | 12.7 $\mu$m |
| Slit Width: | 1 mm |
| Cell Volume: | 5.6 liters (5.64 liters) with calibration loop) |

### C. Miran 1A Calibration

#### 1. Methods

Standards of HCFC-123 in air were prepared using the closed-loop calibration system supplied with the Miran 1A. The volume of the calibration system was 5.64 liters. For this volume, the appropriate amounts of HCFC-123 were determined by calculation, and then injected into the calibration system using a Pressure-Lok® Series A-2 1.0-ml gas syringe which was maintained on ice to prevent excessive volatilization of the test article. The following formulae were used to calculate standard concentrations for calibration:

$$\frac{\text{ml HCFC-123 (liquid)} \times \text{Specific Gravity (g/ml)}}{\text{Molecular Weight (g/mol)}} = \text{moles HCFC-123}$$

$$\text{mol HCFC-123} \times 24.45\ \text{l/mol} \times 1{,}000\ \text{ml/l} = \text{ml HCFC-123 (gas)}$$

$$\frac{\text{ml HCFC-123 (gas)}}{\text{Loop Volume}} \times 10^6 = \text{concentration of standard (ppm)}$$

where:

| | |
|---|---|
| Specific Gravity = | 1.46 g/ml |
| Molecular Weight = | 152.93 g/mol |
| Calibrator Volume = | 5640 ml |
| ppm = | Parts per million |

Five standards were prepared in triplicate once during method development and once on the day of exposure. The average absorption (X) was plotted against concentration (Y) to construct a calibration curve, using the linear regression routine in a Hewlett-Packard calculator model HP-20S. The concentration of each atmosphere sample was calculated from this curve.

2. Results

Mean calibration responses were consistent over the test period. Calibration data for method development and animal exposure are presented in Table 2.

## II. EXPOSURE METHODS

### A. Inhalation Exposure System Description

The test atmospheres were generated in a 1.5 $m^3$ (1500-liter) glass and stainless steel whole body inhalation chamber (NYU Type). The chamber was operated at approximately 12-15 air changes per hour (approximately 300-375 liters per minute for a 1500-liter chamber). A HEPA filter and an activated carbon cartridge filter were used to treat room temperature air before it entered the chamber. Mass airflow through the chamber was monitored by a Dwyer Mangehelic® Indicating Transmitter pressure gauge. Negative pressure within the chamber was monitored by a Dwyer Mangehelic® Indicating Transmitter. Treatment of exhaust air consisted of drawing the air through an activated carbon bed, a HEPA filter and a water-spray fume scrubber. A Solomat Sensor and transmitter was used to monitor temperature and relative humidity. Oxygen content was measured by an Enmet oxygen meter.

Temperature, relative humidity, oxygen content, mass airflow and negative pressure within the chamber were continually monitored and recorded every 30 minutes through the use of Labtech Notebook Data acquisition software, a Packard Bell PB286 computer and a Brother M-1824L printer.

### B. Method of Test Atmosphere Generation

The HCFC-123 atmospheres were generated at a concentration of 10,000 ppm by passing a flow of dry, compressed air across the surface of the liquid test material contained in a 3000-ml three-necked flask which was partially submerged in a water bath. The water bath temperature was maintained at approximately 45°C to maintain the test material temperature at approximately 20°C (as indicated by a mercury thermometer placed in the liquid test material). An airflow of approximately 6 lpm (as measured by a Sierra Instruments Top=Trak™ Digital Flowmeter) was used to deliver the test material to the chamber via 1/4" O.D. stainless steel lines.

## III. EXPOSURE PERIOD CONDITIONS

### A. General Conditions of Exposure

#### 1. Exposure Duration

Three animals were exposed to the test atmosphere, one animal at a time. The first animal was exposed for 37 minutes from initiation of exposure (26 minutes after a $T_{99}$ of 11 minutes). The second animal was exposed for 67 minutes (60 minutes after a $T_{99}$ of 7 minutes) and the third animal was exposed for 72 minutes (60 minutes after a $T_{99}$ of 12 minutes).

#### 2. Animal Placement

The animals were restrained using a sling apparatus which was placed in the approximate center of the $1.5m^3$ whole body inhalation chamber.

### B. Inhalation Chamber Environmental Data

#### 1. Methods

Chamber temperature, relative humidity, oxygen content, negative pressure and mass airflow were monitored continually during exposure using the Labtech Notebook Data Acquisition System. Values for these parameters were recorded every five minutes.

#### 2. Results

Chamber temperature, relative humidity, oxygen content, negative pressure and mass airflow means for exposure periods only are presented in Table 3.

### C. Test Atmosphere Characterization

#### 1. Analyzed Concentrations

##### a. Methods

Test article concentration was monitored continuously by drawing a portion of test atmosphere through 1/4" O.D. stainless steel tubing to the Miran 1A Gas Analyzer. The analyzer's pump was used to transport the atmosphere samples. Atmosphere concentration was determined by reading and recording the infrared absorbance value at a given time, and entering this value into the linear regression routine to determine the concentration of the test atmosphere in parts per million (ppm).

b. Results

A concentration reading was generally recorded every five minutes during exposure. Individual concentration data are presented in Table 1.

APPENDIX B (TABLE 1)

PROJECT NO.:WIL-227001 ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION

TEST ATMOSPHERE CONCENTRATION ANALYSIS RESULTS

TARGET: 1% (10,000 PPM)

| ANIMAL NUMBER: | 2154 | | 2242 | | 2276 | |
|---|---|---|---|---|---|---|
| | TIME (HH:MM) | CONCENTRATION (PPM) | TIME (HH:MM) | CONCENTRATION (PPM) | TIME (HH:MM) | CONCENTRATION (PPM) |
| | 00:11* | 9907 | 00:07* | 10,008 | 00:12* | 9907 |
| | 00:14 | 10,412 | 00:10 | 10,008 | 00:15 | 11,020 |
| | 00:20 | 9805 | 00:15 | 10,311 | 00:20 | 10,716 |
| | 00:25 | 10,615 | 00:20 | 10,109 | 00:25 | 10,109 |
| | 00:30 | 9907 | 00:25 | 10,008 | 00:30 | 10,109 |
| | 00:35 | 10,008 | 00:30 | 10,109 | 00:35 | 10,109 |
| | 00:37 | GENERATION STOPPED | 00:35 | 10,109 | 00:40 | 10,008 |
| | | | 00:40 | 10,008 | 00:45 | 10,210 |
| | | | 00:45 | 9907 | 00:50 | 10,210 |
| | | | 00:50 | 10,109 | 00:55 | 9805 |
| | | | 00:55 | 10,008 | 01:00 | 9805 |
| | | | 01:00 | 9907 | 01:05 | 10,109 |
| | | | 01:05 | 10,008 | 01:10 | 10,008 |
| | | | 01:07 | GENERATION STOPPED | 01:12 | GENERATION STOPPED |
| MEAN : | | 10,109 | | 10,047 | | 10,163 |
| S.D. : | | 326.2 | | 105.4 | | 344.8 |
| C.V.(%): | | 3.23 | | 1.05 | | 3.39 |
| N : | | 6 | | 13 | | 13 |

* = T99

PROJECT NO.:WIL-227001

APPENDIX B (TABLE 2)
ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION
MIRAN 1A CALIBRATION DATA

PATHLENGTH = 0.75 METERS | SLIT = 1 mm | WAVELENGTH = 12.7 MICRONS

| CALIBRATION DATE | STANDARD CONCENTRATIONS | ABSORBANCE (a) | R VALUE |
|---|---|---|---|
| 03/28/94 | 4,139 PPM | 0.051 | |
| | 8,277 PPM | 0.096 | |
| | 12,416 PPM | 0.137 | 0.9994 |
| | 16,555 PPM | 0.176 | |
| | 20,693 PPM | 0.214 | |
| 04/05/94 | 4,139 PPM | 0.051 | |
| | 8,277 PPM | 0.097 | |
| | 12,416 PPM | 0.138 | 0.9992 |
| | 16,555 PPM | 0.178 | |
| | 20,693 PPM | 0.215 | |

(a) VALUES GIVEN IN ABSORBANCE UNITS. EACH IS THE MEAN OF THREE TRIALS.
mm = MILLIMETERS PPM = PARTS PER MILLION

PROJECT NO.:WIL-227001

APPENDIX B (TABLE 3)
ACUTE PHARMACOTOXIC STUDY OF HCFC-123 IN DOGS BY INHALATION
CONDITIONS OF EXPOSURE (MEANS)

CONDITIONS FOR 1.5 M3 WHOLE BODY EXPOSURE CHAMBER — EXPOSURE DATE: 04/05/94

| TEMPERATURE (DEGREES C) | HUMIDITY (%RH) | OXYGEN CONTENT (%) | MASS AIRFLOW (SLPM) | NEGATIVE PRESSURE (IN. H2O) |
|---|---|---|---|---|
| 24.2 | 33 | 20.7 | 307 | 0.53 |

C = CELSIUS  RH = RELATIVE HUMIDITY  SLPM = STANDARD LITERS PER MINUTE  IN. H2O = INCHES OF WATER

Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

# APPENDIX C

## Additional Validation Work

## ADDITIONAL METHOD VALIDATION (3-2-94)

At the Sponsor's request, additional method validation work was performed by doing a concurrent calibration of the Miran 1A and the HP 5890 Series II Gas Chromatograph. Standards were prepared in the closed loop calibrator supplied with the Miran 1A. The loop, including the Miran path and pump, has a set volume of 5.64 liters. Standards were prepared by injecting liquid test material through a septum port into the loop. The following table shows the standards produced.

| Total Volume | Volume Added | Standard Concentration |
|---|---|---|
| 0.2 ml | 0.2 ml | 8277 ppm |
| 0.3 ml | 0.1 ml | 12,416 ppm |
| 1.0 ml | 0.7 ml | 41,386 ppm |
| 1.5 ml | 0.5 ml | 62,079 ppm |

These are the same levels that were used for calibration for the animal exposure portion of the study. With the Miran settings the same as well, the calibration was initiated. After each injection into the closed loop calibrator, a 10-ml sample was drawn from the loop and injected into the gas sampling valve of the gas chromatograph, which injected a metered volume of 0.25 ml to the column and subsequently to the detector. Repeating this procedure for each level, the following calibration was obtained.

| | Miran 1A | | Gas Chromatograph | |
|---|---|---|---|---|
| Standard Conc. | Absorbance | Run # | Peak Area | Response |
| 8277 ppm | 0.092 | 1 | 178,578 | 0.046 |
| 12,416 ppm | 0.133 | 2 | 289,562 | 0.043 |
| 41,386 ppm | 0.370 | 3 | 953,707 | 0.043 |
| 62,079 ppm | 0.528 | 4 | 1,403,6866 | 0.044 |
| r = 0.9997 | | Mean: | 0.044 | |
| | | S.D.: | 0.001 | |
| | | C.V.(%): | 3.2 | |

Next, a standard of known concentration was prepared by injecting a known volume of liquid test material into a 1.0-liter Tedlar® bag filled with air. The volume of air in the bag was determined by metering air into the bag at a known flow rate for a known time. Flow into the bag was measured with a Top=Trak™ digital flow meter and timed with a stopwatch. Bag #1 was filled for 1.25 minutes at a rate of 0.92 lpm, which results in a volume of 1.15 liters. To this bag, 50 microliters (0.05 ml) of liquid test material were added to yield a calculated concentration of 10,149 ppm.

Bag #2 was filled for 1.25 minutes at a rate of 0.86 lpm, which results in a volume of 1.075 liters. To this bag, 1.0 ml of the standard prepared above (calculated at 10,149 ppm) was added.

Bag #3 was filled for 1.25 minutes at a rate of 0.89 lpm, which results in a volume of 1.11 liters. To this bag, 10 ml of the standard prepared above (calculated at 10,149 ppm) were added.

Samples of 10 ml were withdrawn from these bags and injected into the gas chromatograph. Between injections, the syringe was heated with a hair dryer for several seconds to prevent carry over from one injection to the next. In addition, the gas sampling loop was flushed with approximately 60 ml of room air between each injection. The results of these injections are presented below.

| Calculated Conc. | Peak Area | Actual Conc. | Mean | S.D. | C.V.(%) |
|---|---|---|---|---|---|
| 10,149 ppm | 241184 | 10,612 ppm | | | |
| 10,149 ppm | 243823 | 10,710 ppm | 10,607 ppm | 105.6 | 1.0 |
| 10,149 ppm | 238170 | 10,499 ppm | | | |

Because the measured concentration is different from the calculated concentration, the subsequent dilutions were calculated based on this actual measured concentration for the high level standard.

Bag #2

| Calculated Conc. | Peak Area | Actual Conc. | Mean | S.D. | C.V.(%) |
|---|---|---|---|---|---|
| 10 ppm | 250 | 12 ppm | | | |
| 10 ppm | 262 | 12 ppm | 12 ppm | 0.0 | 0.0 |
| 10 ppm | 254 | 12 ppm | | | |

Bag #3

| Calculated Conc. | Peak Area | Actual Conc. | Mean | S.D. | C.V.(%) |
|---|---|---|---|---|---|
| 96 ppm | 2069 | 96 ppm | | | |
| 96 ppm | 1998 | 93 ppm | 95 ppm | 1.53 | 1.6 |
| 96 ppm | 2040 | 95 ppm | | | |

## ADDITIONAL METHOD VALIDATION (4-22-94)

At the Sponsor's request, additional validation work was performed after the follow-up experiment. The Miran 1A was calibrated with a single trial using the same standard levels as were used for the whole body exposure portion of the study. Standards were prepared in the closed loop calibrator supplied with the Miran 1A. The loop, including the Miran path and pump, has a set volume of 5.64 liters. Standards were prepared by injecting liquid test material through a septum port into the loop. A copy from raw data of the Miran IA output is given in Figure 1. The following table shows the standard calibration.

| Total Volume | Standard Concentration | Absorbance |
|---|---|---|
| 0.1 ml | 4139 ppm | 0.050 |
| 0.2 ml | 8277 ppm | 0.095 |
| 0.3 ml | 12,416 ppm | 0.136 |
| 0.4 ml | 16,555 ppm | 0.176 |
| 0.5 ml | 20,693 ppm | 0.213 |

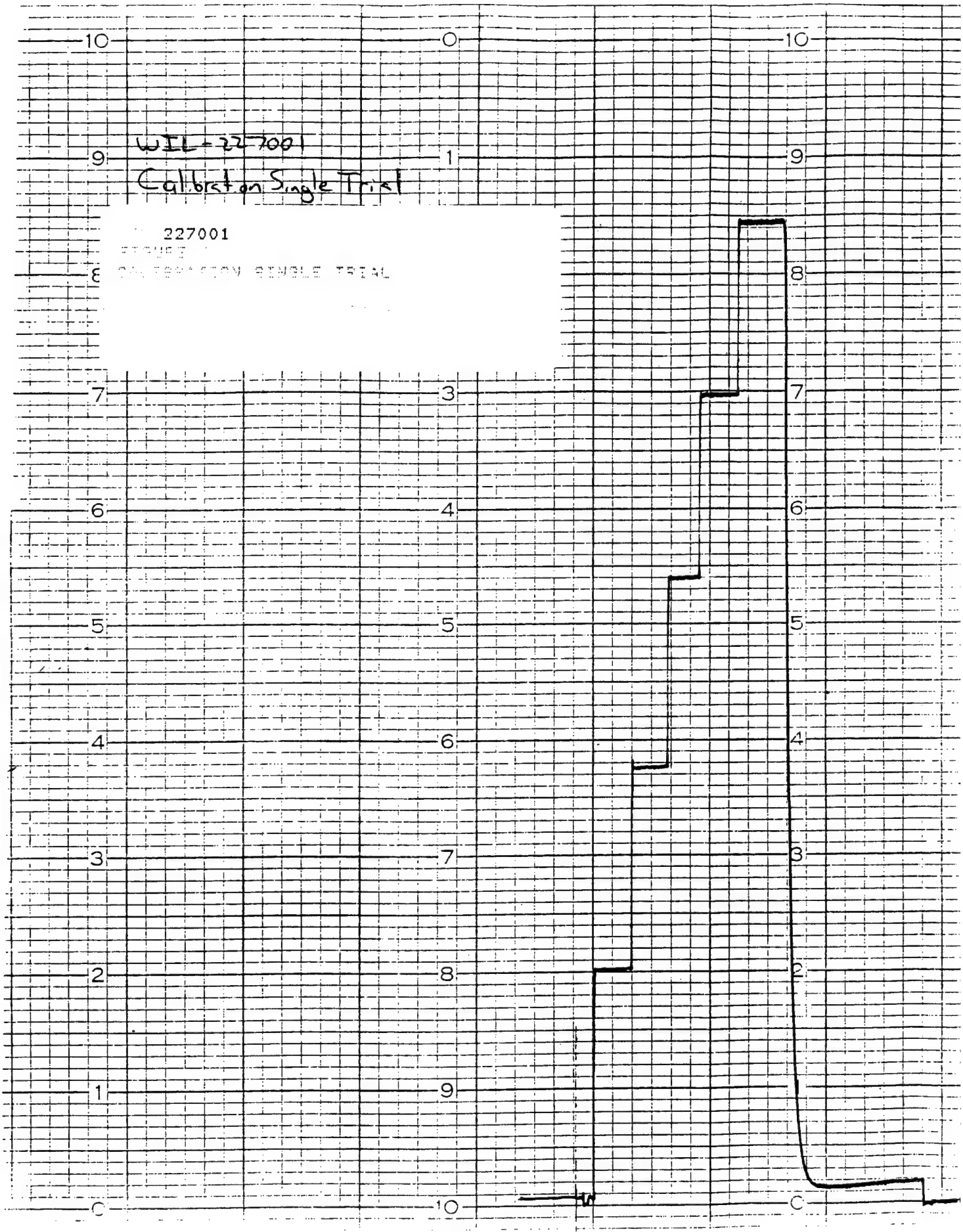
The 100-liter Tedlar® bag provided by the Sponsor was filled with dry, compressed air to a known volume by metering the air through a Sierra Top=Trak™ digital flowmeter for a known length of time (9:00 minutes for all trials). The flow, in lpm, was multiplied by the time required to fill the bag to calculate the total volume of air introduced into the bag. The following formula was used to calculate the concentration of the standards prepared:

$$1\ T.M._{gas} = ml\ T.M._{liquid} \times density\ (g/ml) \times 24.45\ l/mol \div molecular\ weight\ (g/mol)$$

$$Standard\ Concentration\ (ppm) = (1\ T.M._{gas}/Volume\ of\ Air\ in\ Bag) \times 10^6$$

A 10.0 ml Pressure-Lok® glass syringe was used to inject the test material through the septum port into the Tedlar® bag. The bag was then connected to the sample line of the Miran 1A and the contents of the bag were drawn through the instrument by the onboard pump and exhausted. Copies from the raw data of the output from the Miran IA are given in Figures 2, 3 and 4. Below is a table showing the results of tests at three levels.

| Bag fill rate (lpm) | Total Volume (liters) | Amount added (ml) | Standard Concentration | Maximum Absorbance | Measured Concentration |
|---|---|---|---|---|---|
| 9.00 | 81.00 | 1.75 | 5043 ppm | 0.062 | 5095 ppm |
| 8.97 | 80.73 | 3.50 | 10,120 ppm | 0.110 | 9976 ppm |
| 9.00 | 81.00 | 7.00 | 20,172 ppm | 0.192 | 18,314 ppm |

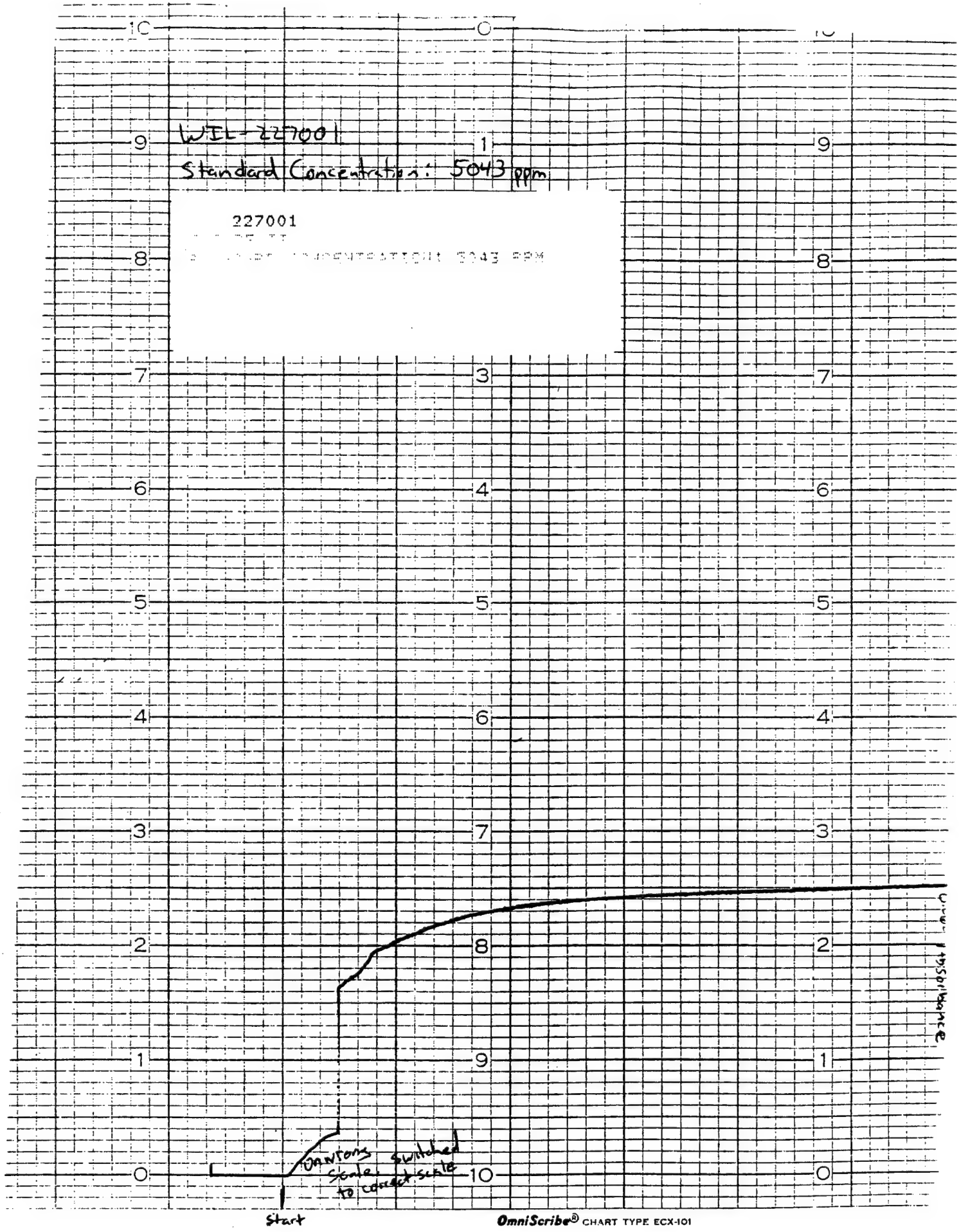

WIL-227001
Calibration Single Trial
227001

WIL-227001

Standard Concentration: 5043 ppm

227001

[illegible] CONCENTRATION 5043 PPM

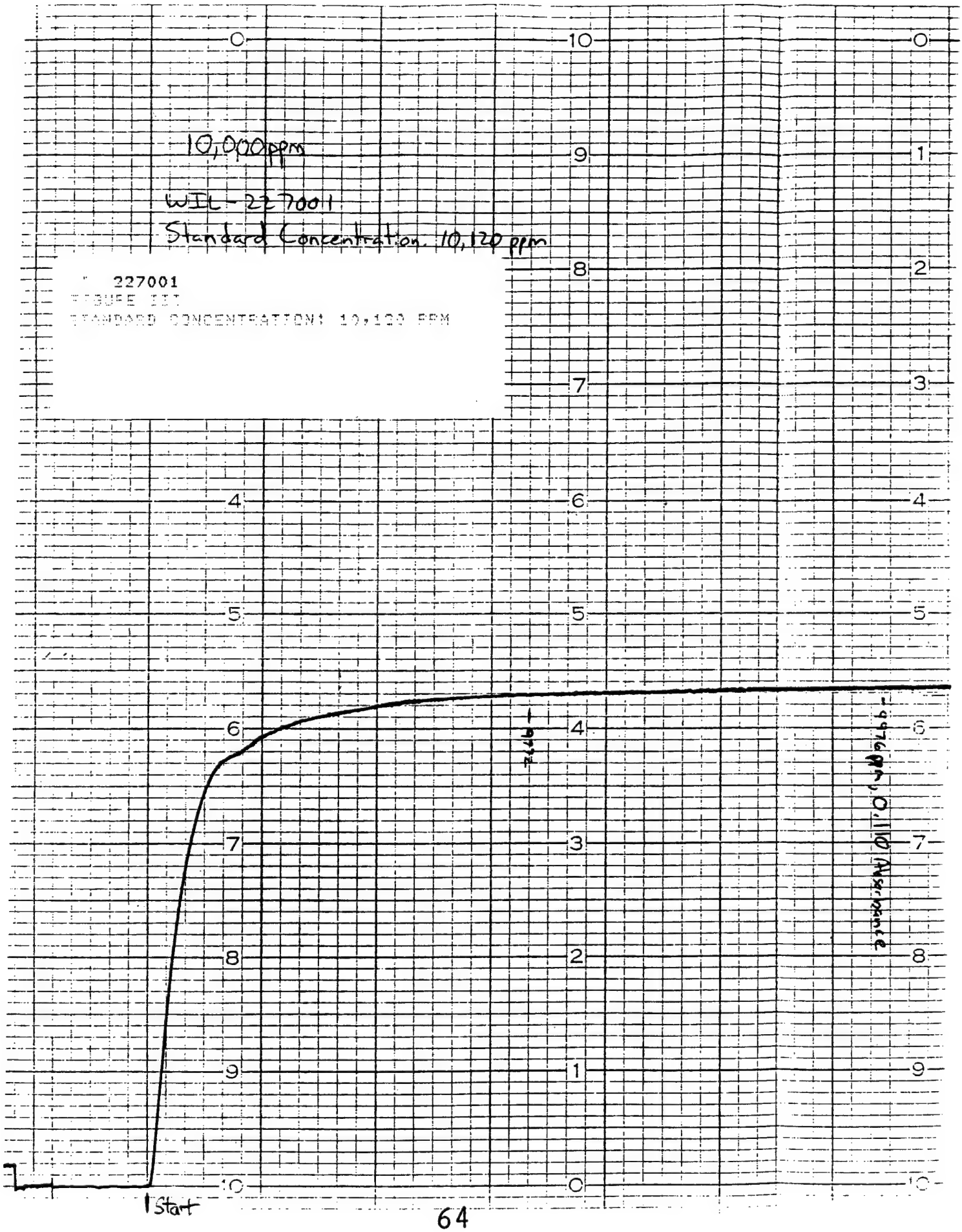


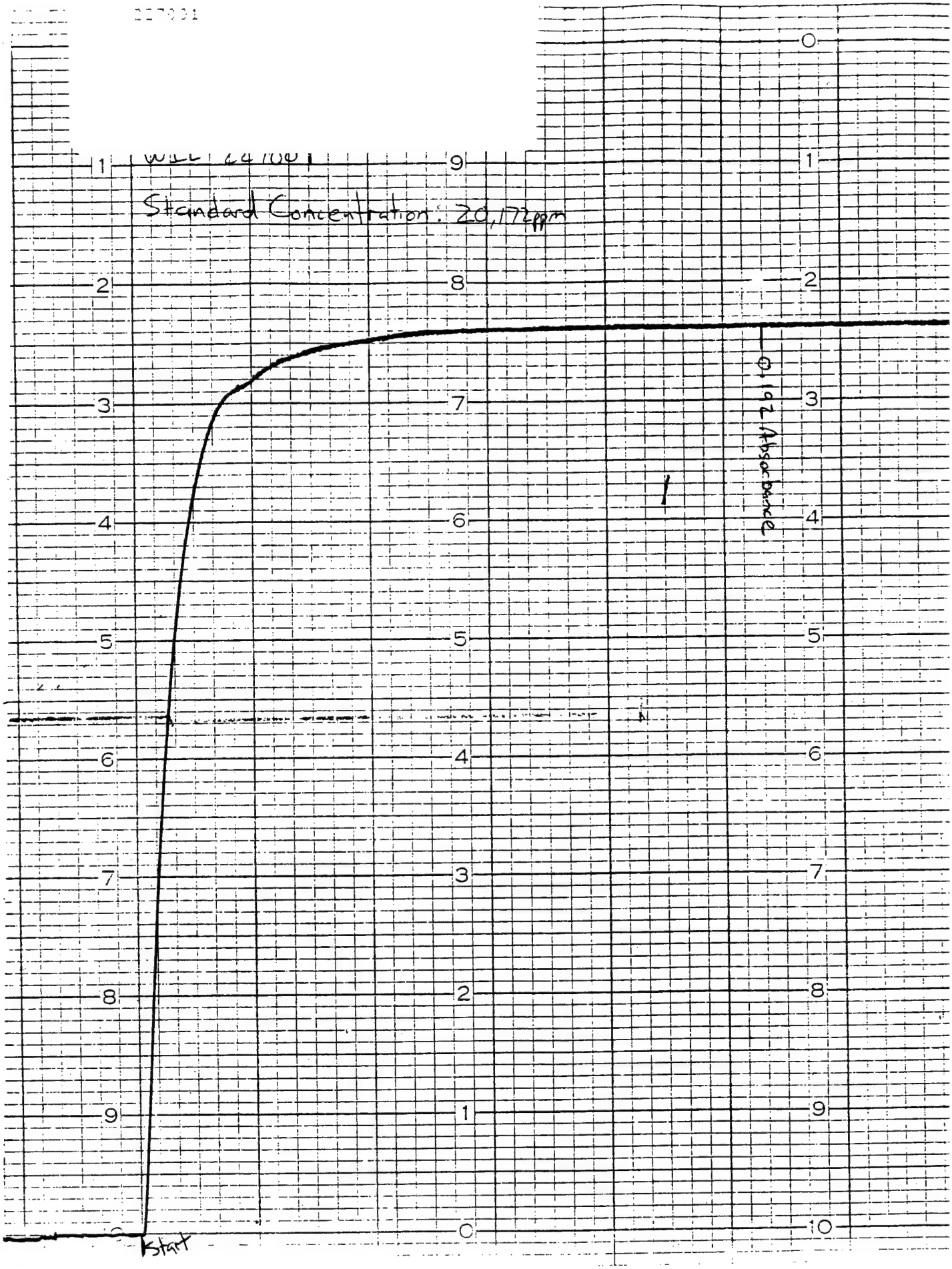
10,000ppm

WIL-227001

Standard Concentration. 10,120 ppm

227001
TUBE III
STANDARD CONCENTRATION: 10,120 PPM

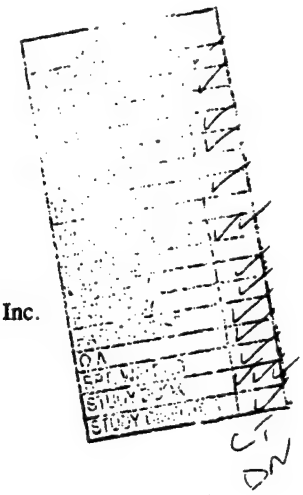



Standard Concentration: 20,172ppm
0.192 Absorbance
Start

Acute Pharmacokinetic Study of
HCFC-123 In Dogs by Inhalation

## APPENDIX D

Study Protocol



Study Number: WIL-227001

**PROTOCOL AMENDMENT II**

Sponsored by: ManTech Environmental Technology, Inc.

A. Title of Study:

Acute Pharmacokinetic Study of HCFC-123 in Dogs by Inhalation

B. Protocol Addition:

Additional experimentation has been requested by the sponsor.

1) III. STUDY SCHEDULE, additional work

A. Estimated Experimental Start Date: April 5, 1994

B. Estimated Experimental Termination Date: April 5, 1994

C. Estimated Proposed Report Date: May, 1994

2) V. TEST SYSTEM

C. Source: Standard U.S.D.A approval supplied

D. Number of Animals: 2 males will be used

3) VII. EXPERIMENTAL DESIGN

D. Organization and Treatment Regimen

In addition to the work described in the original protocol two male dogs will be exposed to 1% HCFC-123 for 60 minutes starting at $T_{99}$ (the time at which target test article concentration is achieved) in a whole-body chamber (1.5 $m^3$). The animals will be accommodated to the restraint sling and exposure chamber during the week prior to exposure. Each dog will be restrained in a sling, a teflon catheter placed in saphenous or cephalic vein, which will be secured in place and locked with heparinized saline (20 IU/ml). The catheter will be attached to an (polyethylene 96 cm) extension set, and exteriorized from the chamber, and appropriately capped. Extension set will be run through a larger bore tygon tube.

4)VIII. PARAMETERS TO BE EVALUATED

E. Blood Test-Article Concentration

In the additional experiment, blood samples will be collected from each dog at 0 (before the start of exposure), 1, 2, 3, 4, 5, 15, 30 and 60 minute of exposure (starting at $T_{99}$). Samples of 0.5 ml will be drawn using a 1 ml plastic syringe. Samples will be injected into sealed head space vials (provided by the sponsor) and returned to the sponsor for analysis.

F. Tissue Collection

Animals on the additional experiment will not be euthanized or necropsied. They will be returned to stock.

C. Reason for Protocol Addition:

Additional Experimentation has been requested by the sponsor.

Approved By:

ManTech Environmental Toxicology
P. O. Box 31009
Dayton, OH 45437-0009

Darol Dodd, Ph.D.,D.A.B.T.
ManTech Environmental Technology, Inc.

3/11/94
Date

Lt. Col. James McDougal, U.S.A.F.,B.S.C.
Director of Research
Toxicology Division OET
Bldg. 79, Area B
WPAFB, OH 45433-7400

11 Mar 94
Date

Prepared By:

WIL Research Laboratories, Inc.
Ashland, Ohio 44805-9281

Christopher P. Chengelis, Ph.D.,D.A.B.T.
Study Director

3/10/94
Date

| COPIES TO | |
|---|---|
| ACCOUNTING | |
| ANALYTICAL | |
| ACOUSTICS | |
| CENTRAL FILE | ✓ |
| CLIN. PATH | ✓ |
| DART LAB | |
| HISTOLOGY | ✓ |
| INHALATION | |
| IN-LIFE MANAGER | ✓✓ |
| METABOLISM | |
| NECROPSY | ✓ |
| PHARMACY | ✓ |
| PATHOLOGIST | ✓ |
| Q.A. | ✓ |
| RPT. WRITING | ✓ |
| STUDY BOOK T&L | ✓✓ |
| STUDY DIRECTOR | ✓ |

CT
DN
IL

Study Number: WIL-227001

**PROTOCOL AMENDMENT I**

Sponsored by: ManTech Environmental Technology, Inc.

A. Title of Study:

Acute Pharmacokinetic Study of HCFC-123 in Dogs by Inhalation

B. Protocol Modification:

1) III. TEST SCHEDULE

A. Experimental Start Date: November 24, 1993

B. Experimental Termination Date: November 24, 1993

C. Report Date January 7, 1994

2) IV. TEST ARTICLE DATA

B. Lot Number: 106684052005

E. Physical Description: Under the conditions of exposure, the test article is a colorless vapor. It was provided in liquid form in unpressurized metal containers.

F. Storage Conditions: Keep at freezer temperatures until the day before use.

3) V. TEST SYSTEM

C. Source: LBL Kennels (a U.S.D.A approved Supplies) Reelsville, IN

F. Identification: Rather than tattoos, animals were uniquely identified by vendor supplied collar tags.

4) VI. SPECIFIC MAINTENANCE SCHEDULE

A. Animal Housing — The sentence, "During regular working hours,... socialization." has been dropped.

5) VII. EXPERIMENTAL DESIGN

A. Animal Receipt and Acclimation — Acclimation will be for a minimum of 7 days prior to exposure.

F. Exposure

2. Sample Collection — Replace "tevlar" with "tedlar". In addition to the collection of expired air, samples will be collected for test article analysis from either of the tedlar bags or from the exhaled breath side of the one-way non-rebreathing valve.

6)VIII. PARAMETERS TO BE EVALUATED

E. Blood Test-Article Concentrations — Samples of 2.0 ml will be drawn and subdivided at 0.1 ml aliquots.

7)VIII. PARAMETERS TO BE EVALUATED

F. Tissue Collection — The sentence "The chest will be opened and... chilled in an ice/water bath" has been deleted. Tissues will be drawn in the following order.

Heart
Fat
GI
Kidney
Liver
Muscle

Duodenum has been added to the tissue list.

C. Reason for Protocol Modification:

1) Schedule for study has been set.

2) More accurate test article information has been received from the client,

3) Test system information was completed.

4) Because of the time involved in accommodating the animals to the exposure slings, regularly scheduling exercise periods were not practical. Exercise was given but not at regularly scheduled periods.

5) The acclimation was shortened in order to decrease the time animals were kept unnecessarily. In the opinion of the WIL study director and veterinarian, a 14 day holding period was not necessary for the study.

6) Schedule change was made to increase flexibility.

7) Preliminary work by the sponsor indicated that chilling the tissue was not necessary. The duodenum was added to the list at the sponsor's request.

Approved By:

ManTech Environmental Toxicology
P. O. Box 31009
Dayton, OH 45437-0009

Darol Dodd, Ph.D.,D.A.B.T.
ManTech Environmental Technology, Inc.

11/24/93
Date

For R. J. Williams, Capt, USAF, BSC
Lt. Col. James McDougal, U.S.A.F.,B.S.C.
Director of Research
Toxicology Division OET
Bldg. 79, Area B
WPAFB, OH 45433-7400

24 Nov 93
Date

Prepared By:

WIL Research Laboratories, Inc.
Ashland, Ohio 44805-9281

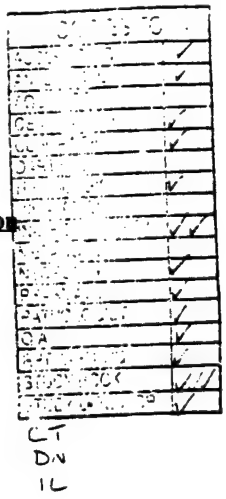
Christopher P. Chengelis, Ph.D.,D.A.B.T.
Study Director

11/24/93
Date

PROTOCOL

Acute Pharmacokinetic Study of HCFC-123 in Dogs by Inhalation

WIL Study No.: WIL-227001



For

Mailing:

ManTech Environmental Technology
P.O. Box 31009
Dayton, OH 45437-0009

Shipping:

Bldg. 79, Area B
Wright-Patterson AFB, OH 45433

By

WIL Research Laboratories, Inc.
Ashland, Ohio 44805-9281

October 15, 1993

## ACUTE PHARMACOKINETIC STUDY OF HCFC-123 IN DOGS BY INHALATION

WIL Study No.: WIL-227001

### I. OBJECTIVE OF STUDY

The objective of this study is to evaluate the pharmacokinetic behavior of the test article resulting from single acute exposure by inhalation to dogs and use the data to support the development of a PBPK model. The biological phase (animal procurement, training, exposure, sample collection, etc.) will be conducted by WIL Research Laboratories. ManTech will complete the analysis. This protocol describes only those portions of the study for which WIL Research Laboratories is responsible.

The study will be conducted in compliance with the EPA (Environmental Protection Agency) Good Laboratory Practice Standards, 40 CFR Part 792.

### II. PERSONNEL INVOLVED IN THE STUDY

A. Sponsor Study Monitor

Darol Dodd, Ph.D., D.A.B.T.
ManTech Environmental Technology, Inc.
Dayton, OH
Telephone: (513) 256-3600, ext. 214

B. WIL Study Director

Christopher P. Chengelis, Ph.D., D.A.B.T.
Associate Director of Toxicology

C. WIL Toxicology Department Responsibilities

1. E. Crosby Tompkins, Ph.D., D.A.B.T.
   Vice President, Director of Toxicology
2. Dennis J. Naas, B.S.
   Assistant Director of Toxicology
3. Jerry E. Bennick
   Manager, Inhalation Toxicology
4. Lisa Simon, B.S., M.T.(ASCP)
   Supervisor of Clinical Pathology
5. Stanley E. Kopp
   Systems Manager
6. Sally A. Keets, A.S.
   Manager of In-Life Facilities

II. PERSONNEL INVOLVED IN THE STUDY (continued)

7. Deborah L. Little
   Manager of Quality Assurance

8. Kerin Clevidence, B.S.
   Acting Section Head I - Pathology and
   Developmental Toxicology Laboratory

9. Gary R. Kiplinger, B.S.
   Associate Toxicologist

10. Kevin D. Oberholtzer, B.S.
    Manager of Technical Report Writing

11. Robert R. Dahlgren, D.V.M., Ph.D.
    Diplomate A.C.V.P.
    Director of Pathology

12. Chris Nelson
    Acting Section Head I - Pharmacy

III. STUDY SCHEDULE DATA

A. Proposed Experimental Start Date: To be added by amendment

B. Proposed Experimental Termination Date: To be added by amendment

C. Proposed Report Date: To be added by amendment

IV. TEST ARTICLE DATA

| | |
|---|---|
| A. Identification: | HCFC-123 |
| B. Lot Number: | To be provided by Sponsor. |
| C. Purity: | To be provided by the Sponsor. |
| D. Stability: | To be provided by the Sponsor. |
| E. Physical Description: | Under conditions of exposure, test article is a colorless gas. It will be provided in liquid form in a pressurized cylinder. |
| F. Storage Conditions: | To be provided by Sponsor. |

IV. TEST ARTICLE DATA (continued)

G. Reserve Samples: Retention sample(s) will be collected and stored in accordance with standard operating procedures.

H. Personnel Safety Data: Organic respirator and eye protection are required during periods when direct exposure to the gas is likely to occur. An MSDS will be requested.

V. TEST SYSTEM

A. Species: Dog

B. Strain: Beagle

C. Source: Standard U.S.D.A. - approved supplier.

D. Number of Animals: Approximately 16 males will be purchased.

E. Approximate Age and Body Weight: Animals will be approximately 12 to 14 months of age when received. All study dogs will be within 5% of the mean body weight.

F. Identification System: Animals will be uniquely identified by an ear tattoo. Individual cage cards will be affixed to each cage and will display the animal number and study number.

G. Rational for Selection:

The dog is the animal model of choice for cardiac sensitization testing with chlorofluorocarbons and chemical candidate for their replacement. This study will determine select pharmacokinetic parameters associated with standard cardiac sensitization in the dog for HCFC-123.

VI. SPECIFIC MAINTENANCE SCHEDULE

A. Animal Housing

All animals will be housed individually in clean suspended stainless steel cages in an environmentally controlled room. The cages will be elevated above stainless steel flush pans which will be cleaned daily. During regular working hours, dogs will be given frequent and regular opportunities for exercise and socialization.

VI. SPECIFIC MAINTENANCE SCHEDULE (continued)

B. Environmental Conditions

Controls will be set to maintain temperature at 72° ± 4°F and relative humidity at approximately 30-70%. Fluorescent lighting will provide illumination for 12 hours per day.

C. Drinking Water

Tap water will be available *ad libitum*. Filters servicing the automatic watering system will be changed regularly according to Standard Operating Procedures. Municipal water supplying the laboratory will be analyzed periodically for contaminants according to Standard Operating Procedures to ascertain that none are present at concentrations that would be expected to affect the outcome of the study.

D. Diet

Approximately 400 g of Purina® Certified Canine Diet #5007 (pellet) will be offered daily for approximately 1-2 hours. Analyses of the certified feed for the presence of contaminants will be provided by the manufacturer to ensure that none are present at concentrations that would be expected to affect the outcome of the study.

VII. EXPERIMENTAL DESIGN

A. Animal Receipt and Acclimation

Each animal will be inspected by qualified personnel upon receipt. Dogs judged to be in good health and suitable as test animals will be acclimated for at least 14 days. Dogs will be weighed upon receipt, and in the event of a delay in study start, and twice per month thereafter until study start. During the acclimation period, each dog will be observed twice daily for changes in general appearance and behavior. Immunizations prior to arrival of the dogs will include: distemper, hepatitis, leptospirosis (DHL) Parvo and parainfluenza. Stool samples will be collected during the acclimation period and checked for parasites. Each dog will be given an ECG evaluation.

During the two weeks prior to the start of the study, each dog will be placed in a sling and exposure mask for 15 to 45-minute, graduated daily periods, in order to acclimate them to test article exposure conditions.

B. Randomization

At the conclusion of the acclimation period, animals judged to be suitable for testing will be assigned to the study based on health, body weight, temperament and pretest ECG results. Suitable animals in excess of the number needed will be randomly chosen for exclusion.

VII. EXPERIMENTAL DESIGN (continued)

C. Route and Rationale of Test Article Administration

The test article will be given by inhalation exposure as this is the most likely route of exposure for the general population.

D. Organization and Treatment Regimen

| HCFC-123 Conc. (%, v/v) | Duration of Exposure 1 min | 5 min | 60 min | 60 min + 30 min recovery |
|---|---|---|---|---|
| 1 | N = 2 | N = 2 | N = 2 | N = 2 |
| 5 | N = 2 | N = 2 | NA | NA |

E. Probe Study

Prior to the initiation of the main study a probe study will be conducted. The purpose will be to determine the appropriateness of the exposure concentrations and refine techniques to be used on the main study. Two males will be carried through the exposure system and necropsy as described below. One dog will be exposed for 5 minutes to 5% HCFC-123, the other will be exposed for 10 minutes to 1% HCFC-123.

ECGs will be recorded (Section VIII.D) and Blood samples collected (Section VIII.E).

Any samples collected will be provided to the sponsor to use at their discretion.

F. Exposure

1. Exposure Methods

All animals will be exposed in a nose-only inhalation exposure system operated under dynamic conditions to sustain air flows of approximately 10 air changes per hour, ensuring an adequate oxygen content of approximately 19 percent or above and an evenly distributed exposure atmosphere. Each dog will be fitted with a rubber snout mask fitted with a non-rebreathing valve. An appropriate generation/exposure system consisting of a modified 500 L whole body inhalation chamber equipped with connection ports, will be devised prior to the animal exposures. Wherever possible, tubing and connection will be made of stainless steel or brass. The methods of generation and exposure will be documented in the study records and described in detail in the final report. Exposure will be initiated when the chamber is at target concentration. Oxygen may be supplemented as needed to maintain a level of approximately 19% or above. One or two dogs per exposure period will be exposed. Feed and water will be withheld during the exposure period. Controls will be set to maintain exposure atmosphere temperature at approximately 22°C (± 2°) and humidity between approximately 40 to 60 percent.

VII. EXPERIMENTAL DESIGN (continued)

2. Sample Collection

During the exposure period, samples of expired air will be collected in appropriate sized tevlar bags.

| | |
|---|---|
| 1 & 5 minute exposures: | entire period |
| 60 minute exposure: | for 5 minute periods starting at 10, 25, 40 and 55 minutes of the exposure periods |
| 30 minute recovery: | at 10 and 25 minutes post-exposure |

An appropriate method of determining the volume of air expired will be developed and detailed in the raw data and final report. Data will be used to calculate minute volumes.

G. Test Atmosphere Monitoring

Exposure concentrations within each chamber will be monitored by infrared techniques. The method of analysis used will be documented in the study records and described in detail in the final report.

Mass air flow, oxygen content, temperature and humidity shall be monitored continuously and recorded approximately every 30 minutes during the periods of atmosphere generation. Test atmosphere concentrations at or near the point of inspiration will be determined during pre-study method development and confirmed, if necessary, during the animal exposures.

VIII. PARAMETERS TO BE EVALUATED

A. Viability Checks

All animals will be checked for mortality and moribundity each morning and afternoon.

B. Clinical Observations

The dogs will be observed for clinical signs of toxicity continually during the exposure period; signs of toxicity, if present, will be recorded as they are observed. Documentation of animals without signs will be recorded in a general comment.

C. Individual Body Weights

Individual body weights will be recorded weekly during the pre-treatment period and immediately before each exposure period for each dog.

VIII. PARAMETERS TO BE EVALUATED (continued)

D. Electrocardiographic Examinations

Pretreatment ECG's will be recorded with a Vetronics EKG analyzer. Only modified lead 1, 2, 3 electrocardiograms will be collected. Alligator clip electrodes will be placed on each limb.

During the probe study, exposures, ECGs will be collected continuously with a Cambridge model 3035/2 three channel electrocardiograph chart speed will be 10 mm/sec and response sensitivity will be set at 5 mm/mV.

E. Blood Test-Article Concentrations

Prior to initiation of exposure, dogs will be fitted with 2" 20g teflon catheter (saphaneous or cephalic vein) and stainless steel three-way valve or other appropriate blood collection apparatus which may be heparinized. Blood samples will be taken at both exposure concentrations, as follows:

| Exposure Period | Sample Times |
|---|---|
| Probe Study: | |
| 5 minute | 0, 1, 2, 3, 4, 5 |
| 10 minute | 0, 1, 2, 3, 4, 5, 7.5, 10 minutes |
| Main Study: | |
| 1 minute | 0, 1 minutes |
| 5 minutes | 0, 1, 2, 3, 4, 5 minutes |
| 60 minutes | 0, 1, 2, 3, 4, 5, 7.5, 10, 15, 30, 45, 60 minutes |
| 30 min. recovery | 2, 5, 15, 30 minutes post-exposure |

Samples will be of approximately 0.5 ml, drawn by glass syringe. Each sample will be subdivided into three approximate 0.15 ml subsamples prepared by injection into preweighed, sealed vials. A precise weight for each subsample will be determined. Each vial will be labeled with study number, draw time and replicate number. The samples will be placed on dry ice and transferred to the sponsor for analysis. Vials will be supplied by the Sponsor.

F. Tissue Collection

Animals will be euthanized with a combination of barbiturate and potassium chloride immediately after the final blood sample is taken. The necropsy room will be kept at 20-24°C. The chest will be opened and the cavity chilled (either liquid nitrogen or ice-packs), the following organs will be removed (in the order indicated), placed in plastic bags, and then chilled in an ice/water bath.

VIII. PARAMETERS TO BE EVALUATED (continued)

Heart
Skeletal muscle (thigh)
Liver
Kidney
Fat

After sufficient time has passed for chilling to be completed, tissue will be removed and three small subsamples, each weighing approximately 500 mg, will be taken. Each will be placed in preweighed sample vial, and then a precise weight taken vial will be labeled with study number, animal number, tissue and replicate number. Vials will be proved by the sponsor. Upon sealing, the vials will be placed on dry ice and transferred to the sponsor along with the most recent body weights.

IX. STATISTICAL METHODS

Not applicable.

X. QUALITY ASSURANCE

The study is considered to be exempt, and will not be audited by the WIL Quality Assurance Unit while in progress to assure compliance with Good Laboratory Practice regulations, adherence to the protocol and to WIL Standard Operating Procedures.

This study is not a regulated study and will not be included on the master schedule.

XI. RECORDS TO BE MAINTAINED

All original raw data records will be stored in the Archives at WIL Research Laboratories, Inc. Exposure and sampling times will be carefully recorded and reported.

XII. WORK PRODUCT

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including raw paper data, magnetically encoded records and specimens will be retained at no charge for a period of six months following issuance of the final report in the Archives at WIL Research Laboratories, Inc. Thereafter, WIL Research Laboratories will send all work product to the sponsor.

XIII. REPORTS

The final report will contain a summary, test material data, detailed methods and procedures, and a complete inventory of samples taken and their weights or volumes, as appropriate.

## XV. PROTOCOL MODIFICATION

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director.

## XIV. ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

1. The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that the study described in this protocol does not unnecessarily duplicate previous experiments.

2. Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory standard operating procedures.

3. Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action in as timely a manner as possible.

XIV. ANIMAL WELFARE ACT COMPLIANCE (continued)

4. Methods of euthanasia used during this study are in conformance with the above referenced regulation.

XVI. PROTOCOL APPROVAL

ManTech Environmental Toxicology
P.O. Box 31009
Dayton, OH 45437-0009

Darol Dodd, Ph.D., D.A.B.T.
ManTech Environmental Technology, Inc.

10/21/93
Date

WIL Research Laboratories, Inc.
Ashland, Ohio 44805-9281

Christopher P. Chengelis, Ph.D., D.A.B.T.
Study Director

10/15/93
Date

Lt. Col. James McDougal, U.S.A.F., B.S.C.
Director of Research
Toxicology Division OET
Bldg. 79, Area B
WPAFB, OH 45433-7400

10/21/93
Date